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TITLE: Vaccination of High-Risk Breast Cancer Patients with Carbohydrate Mimicking Peptides

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**14. ABSTRACT**
The expression of the Tumor Associated Carbohydrate Antigens such as the neolactoseries antigen Lewis Y (LeY) and gangliosides such as GM2 and GD2/GD3 are amplified on breast cancer cells and is linked to poor prognosis and high risk of disease relapse. Immunotherapy to direct responses to TACA is, therefore, perceived to be of clinical benefit. To overcome this deficiency, we developed mimotopes of TACA to induce more robust cross-reactive and tumor-specific responses. In preclinical studies, immunization with these mimotopes reduce tumor burden and inhibited metastatic outgrowth of murine tumor cells expressing TACA structural homologues. Thus, peptide mimotopes of TACA represent a new and very promising tool to induce a strong immune response to TACA expressed on Breast Cancer cells. Based on encouraging preclinical results, our objectives are for the current funding period (years 1 and 2) are to develop the necessary preclinical data required by the Food and Drug Administration (FDA) for filing an Investigational New Drug (IND). In this context we: 1.) Developed the necessary procedures for the required Good Laboratory Practice (GLP) studies; 2.) Defined problems in scale up of the manufactured mimotope vaccines; 3.) Identified alternative mimotopes of TACA that circumvent the scale up problems.

**15. SUBJECT TERMS**
1.) Developed the necessary procedures for the required Good Laboratory Practice (GLP) studies; 2.) Defined problems in scale up of the manufactured mimotope vaccines; 3.) Identified alternative mimotopes of TACA that circumvent the scale up problems.

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**Introduction**

Carbohydrates are the most abundantly expressed self-antigens on tumor cells and consequently they are perceived as viable targets for immunotherapy. Examples of tumor-associated carbohydrate antigens include the gangliosides GM2, GD2, GD3, and fucosyl GM1, Globo H, polysialic acid, STn and the neolactoseries antigens sialyl-Lewis x (sLex), sialyl-Lewis a (sLea) and Lewis Y (LeY). A major approach to induce responses to these tumor associated carbohydrate antigens (TACA) is carbohydrate-conjugate vaccines. Representative examples of these vaccines in clinical development include those directed toward gangliosides, polysialic acid, Globo, Lewis Y (LeY), and the STn antigen. Because TACA are T-cell–independent antigens and self-antigens, conjugation to immunologic carrier protein is perceived essential to recruit T cell help for antibody generation. Conjugation of TACA does not, however, ensure an increase in immunogenicity because conjugation strategies do not uniformly enhance carbohydrate immunogenicity.

Carbohydrate Mimetic Peptides (CMPs) functioning as T cell dependent surrogates of TACA are proposed to augment responses to TACA. Our rational is based upon our preclinical studies that these surrogate antigens, also referred to as mimotopes, induce immune responses that inhibit tumor growth in animal models. We observe that the analog CMPs 106 (GGIYWRYDIYWRYDIYWRYD) and 107 (GGIYYRYDIYYRYDIYYRYD) mimic several types of carbohydrates on breast cancer cells, principle among them is the LeY antigen. Our purpose is to induce TACA reactive antibodies in breast cancer patients by using a mimotope. We expect to observe after vaccination a robust anti-TACA response in individuals that should positively impact on tumor recurrence.

**Body**

The major goals of this application are to determine the safety and tolerability of immunization with a CMP imunogen; and to determine whether immunization with the CMP generates an immune response against TACAs and TACA expressing breast cancer cell lines.

We have defined three specific aims for a period of five years. As this progress report only concerns Aim 1 subtask 1-5 and Aim 2 subtask 1, because we are still in the preclinical evaluation phase, we will only emphasize our progress in Aim1 subtasks 1-5 and Aim 2 subtask 1. All other aims will be listed in future reports.

**A. Aim 1. Conduct and report preclinical studies relevant for IND filing (Year 1-2)**

Subtask 1: Develop preclinical plan, obtain FDA buy-in, and initiate preclinical studies

Subtask 2: Manufacture preclinical supplies of vaccine, fill/finish, stability monitoring and QA

Subtask 3: Complete animal safety/toxicity studies

Subtask 4: Prepare technical reports for in-house preclinical studies

Subtask 5: Prepare and submit pre-IND and IND documents to the FDA

**B. Aim 2. Assure safety and determine Sufficient Immunogenic Dose (SID) of a mimotope vaccine in a Phase IA dose-escalation trial (year 2-3)**

Subtask 1: Complete GMP manufacture, and obtain FDA and IRB approval prior to initiation Phase 1A

Subtask 2: Enroll and treat 18 patients (maximum) for Phase 1A safety dose-finding study

Subtask 3: Complete safety monitoring of mimotope vaccine

Subtask 4: Determine if SID has been met or repeat dose-finding for OID

Subtask 5: Prepare clinical study report for Phase 1A

**C. Aim 3. Conduct a Phase IB trial to evaluate the immunogenicity of the mimotope vaccine in breast cancer patients (Year 4-5)**

Subtask 1: Enroll and treat a total of 40 patients for Phase IB trial.

Subtask 2: Complete immunogenic analysis of mimotope vaccine

**A. Aim 1 Conduct and report preclinical studies relevant for IND filing (Year 1-2)**

*Subtask 1: Develop preclinical plan, obtain FDA buy-in, and initiate preclinical studies*

The *"Vaccination of High-Risk Breast Cancer patients with Carbohydrate Mimicking Peptide"* grant's second year ended as of April 23, 2008. The second year suffered several set backs in which the study was significantly delayed from moving into its GLP preclinical phase. Within the first year of the study there were a lot of administrative approvals made required for the GLP study. One of the approvals to be made within the first year was to have the submission of approval completed with the Central Arkansas Veterans Healthcare System Research and Development Committee. We were in need of this approval so we could conduct the GLP component of the study in their facilities. The approval process was very time consuming. As of December 17, 2007 (Appendix I) we were approved to use the GLP facilities in the Central Arkansas Veterans Healthcare System. With the approval from the Central Arkansas Veterans Healthcare System, we were still unable to start the GLP preclinical portion of our grant because

we were not informed from the Central Arkansas Veterans Healthcare System that the PI of the project had to obtain a security clearance from the Veterans Administration. We were notified on February 21, 2008 of the security clearance needed for the PI before the study can start. The security clearance is a long unexpected process. We currently have two staff members with security clearances. In the beginning we were only told these two employees were going to need a clearance and they are able to bring a guest over as needed to review the study.

As of May 10, 2008 the GLP protocol has been signed and mice have been ordered for the GLP study and the GLP study has begun. A copy of the signed GLP protocol, the mouse order and the GLP study calendar are in appendices II, III and IV respectively. The pre-clinical portion of the grant is held under Code of Federal Regulation Title 21 Part 58 (21CFR58) Good Laboratory Practice (GLP) for Non-clinical Laboratory Studies. All standard operating procedures (SOPs) are in place and are in accordance with the Code of Federal Regulation Title 21 Part 58 (21CFR58). All SOPs were reviewed for their Annual Review as of April 30, 2008. The staff members' training is in compliance with 21CFR 58. All staff member training is current and is in accordance with 21CFR58 and research administration by-laws of the University of Arkansas for Medical Sciences (UAMS).

*Subtask 2: Manufacture preclinical supplies of vaccine, fill/finish, stability monitoring and QA*

**1. Update on mimicry property of P10s peptide:**

In our previous annual report we presented results on a CMP 106 related CMP analog known as P10s with the WRY amino acid containing sequence WRYTAPVHLGDG which we have linked to the Pan T cell epitope PADRE (PAn-DR Epitope) as the vaccine (WRYTAPVHLGDG-aK-Cha-VAAWTLKAAa). Our previous progress report showed that, the peptide P10s could replace the CMP 106 for the preclinical study. We established that CMP P10s is a viable mimotope for several reasons: (1) P10s binds to BR55-2, an anti-LeY monoclonal antibody; (2) Serum antibodies generated in mice immunized with P10s are capable of cross-reacting with LeY and other breast cell line TACAs and (3) immunization can inhibit tumor growth of Breast Cell lines in vivo. This year we are showing that this anti-tumor activity observed in vivo did not seem to act through complement dependent cytotoxicity (CDC) or apoptotic mechanisms **(Figures 1 and 2)** but act in a novel way by recruiting Natural Killer

(NK) cells to the tumor site (**Figures 3 to 6).**

### 1.1. Effector mechanisms involved in the anti-tumor activity of P10S antibodies

As discussed in our previous report P10s is an analog of the CMP 106 peptide. CMP 106 was described in our original application as the CMP of choice to move forward into the clinic. Because of solubility issues upon scale up to GMP grade (described in previous progress report) we used the fall back CMP P10s. As P10s was shown to induce an efficacious anti-tumor immune response in animals we first investigated the potential killing effect of the P10s anti-sera on tumor cells.

As mentioned in our previous report, immunization with P10s results in an IgM response and since it is well known that IgM are capable of binding complement in a very efficient manner (CDC) we tested sera from immunized C57Bl6 mice against several carbohydrate expressing cells such as EL4 cells and MDA-231 for their ability to mediate complement dependent killing. However, no specific cytotoxicity was detected in these studies **(Figure 1)**.

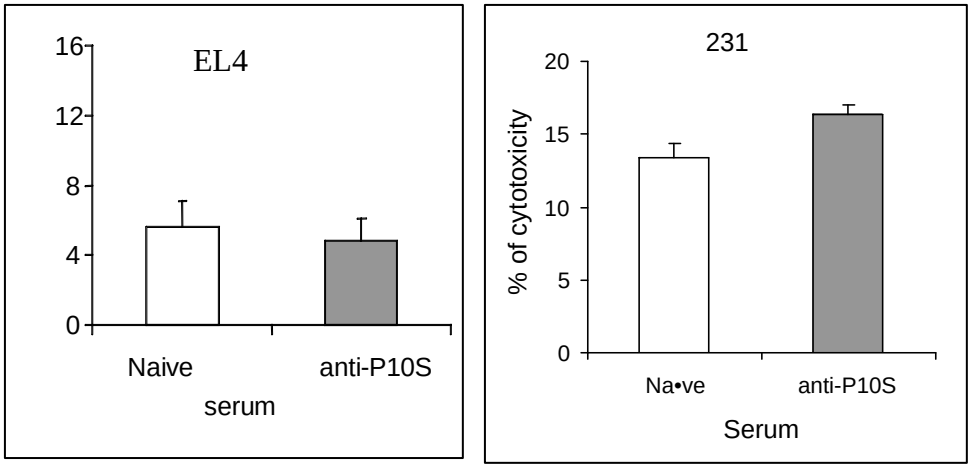



**Figure 1. P10s anti-sera does not mediate CDC against antigen tumor cell lines. MDA-231 and EL4 cells were incubated with 1:50 P10s anti-sera and 1:4 rabbit serum for 4 hours. The number of viable cells was determined and the percentage of cytotoxicity was calculated. The assay was performed in duplicate with medium, sera, and complement controls. Statistics for all three experiments were not significant.**

We showed previously that antibody generated from CMP immunization could induce apoptosis in cancer cells bearing specific carbohydrate antigen (*1*). Therefore, next we tested the capacity of P10s to induce antibodies triggering apoptosis in different cells line including breast cancer cells. However, as shown in **Figure 2,** the sera did not induce apoptosis in the different

tumor cell lines. These observations led us to conclude that the anti-tumor activity observed *in vivo* **(see previous annual report)** was the result of other biological activities than through direct tumor cell killing.

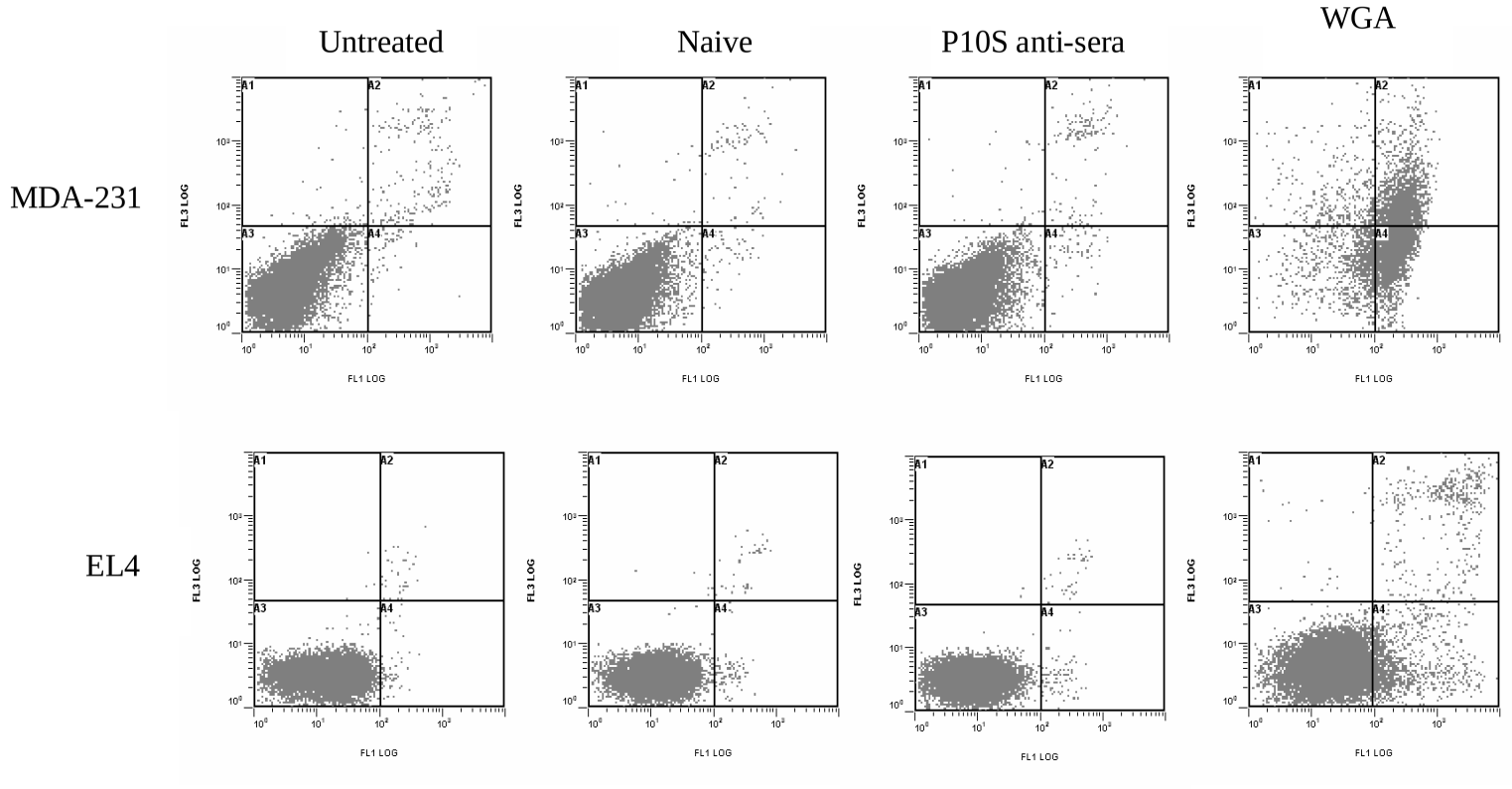


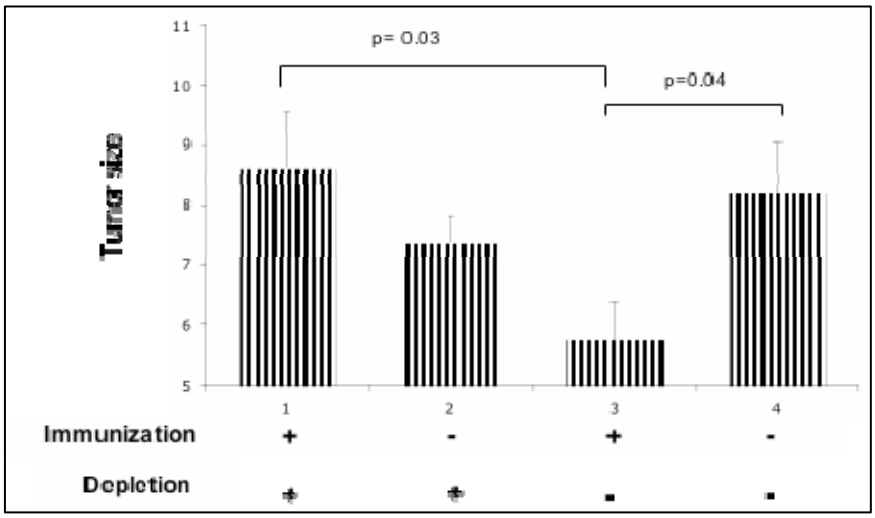

**Figure 2** P10s anti-sera do not induce apoptosis in MDA-231 and EL4

MDA-231 or EL4 cells were incubated overnight with 1/50 P10S anti-sera. Vybrant apoptosis kit no. 3 was used to assess apoptosis. Shown data are representative of four independent experiments. Sera used represent a pool from 5 mice.

Since the immune responses elicited by P10s immunization did not mediate CDC or apoptosis and the responses associated with P10s were thymus independent, we explored the role of other innate immunity mechanisms in the anti-tumor affect. NK cells are known to have a potent anti-tumor activity and thus play an important role in metastasis clearance. In light of the finding of increased NK activation in similar setting by Kawashima et *al.* (2), we hypothesized that the anti-tumor effect observed in mice immunized with P10s could be due to interactions between NK cells and the anti-tumor B cell response that has been boosted by the immunization .

To establish the role of NK cells in the absence of T cells and T dependent antibody responses, athymic mice were xenografted with $2x10^6$ MDA231 cells. This inoculum size was chosen on the basis of previously publish TD50 for this model (3). The mouse strain used, though, proved to be too receptive to the human breast cell tumor line as all mice developed tumors. To evaluate the anti-tumor activity of NK cells in MDA-231 tumor growth, the mice were treated with 25µl of anti-asialo GM1 rabbit serum (to deplete NK cells) four days after the last immunization and two days before tumor inoculation and every 4-5 days until sacrificed, 21 days later. Mice developed palpable tumors one week after inoculation and tumor size were measured 14 days after inoculation using a caliper **(Figure 3).** Mice that were not immunized and untreated with anti-asialo GM1 rabbit serum developed larger tumor than the untreated immunized group confirming our previous results in Balb/c and nude mice ($p=0.04$). Interestingly, mice that were immunized with P10s and depleted of NK cells developed tumors of similar size as the immunized untreated (anti-asialo GM1) control group suggesting that in absence of NK cells the antibodies generated from P10s immunization did not by themselves slow down tumor growth.

**Figure 3 NK cells anti-tumor activity increase after P10s immunization**. Nude mice were inoculated s.c. with 2 x $10^6$ cells MDA-MB-231 cells one week after last immunization. Tumor growth was measured using a caliper and was recorded as the mean of two orthogonal diameters ((a + b)/2). For NK depletion athymic mice were injected intraperitoneally with 25ml of anti-asialo GM1 rabbit serum or normal Rabbit serum two days after the last immunization and four days before MDA-231 tumor cells challenge then every 4-5 days for a month. + means mice were immunized or depleted on NK cells; - means mice were not immunized or not depleted on NK cells.




**This result indicate a relationship between the anti-tumoral effect of NK cell and the humoral response generated against P10s.** Tumor size of the mice immunized with P10s but not depleted of NK cells are significantly (p=0.03) smaller that the immunized and depleted group. The large number of tumor cells in this model was deemed to be above a specific $TD_{50}$ as all mice developed tumors. Consequently, the positive impact of the immunotherapeutic on tumor growth is striking with regards to the otherwise unfavorable conditions of the model. The results confirm that P10s induces a thymus independent humoral immune response capable of activating NK cells that in turn mediate the anti-tumor activity observed**.** It is interesting to note that in their TD50 study Tghian et al. find only 40% decrease in the TD50 for MDA-231 cells in nude mice after whole body irradiation (3). This is an indirect estimate of the contribution of NK cell and B cell function to the tumor surveillance in this model. These results parallel the observed 40% decrease in the tumor size in the depletion model reported here indicating that the

observed effect may represent a major contribution to the innate immunity control of this xenograft model.

The nude mice xenograft model demonstrated the role of the NK cells as the effector cells of an anti-tumor IgM response. In an attempt to further elucidate the nature of the observed cooperation between thymus independent responses and NK cell function, the changes in the tumor environment after P10s immunization was tested next. The Balb/c homologous tumor model was used to verify the generality of the observations in the nude mice model. It has been shown previously that in direct interactions between B cells and NK cells, B cells were able to stimulate the production of IFN$\gamma$ by NK cells and activate the NK cells while NK cells provide help for B cells involved in T cell independent type 2 (TI-2) responses (4). Thus, B cells and NK cells could interact with in an antigen independent manner and the resulting signaling cross-talk may lead to stimulation of both cell types.

To test for a potential role of intratumoral NK/B cell contact for the P10s anti-tumoral effect we tested the effect of P10s immunization on the tumor infiltration by both cell types in the model of 4T1 mouse mammary carcinoma. To this end, 30 days after 4T1 breast cells inoculation, P10s immunized Balb/c mice and unimmunized control groups were sacrificed and the tumors were excised. Tumor tissue sections from these mice were stained with an anti-CD45R (B220, eBioscience) antibody and with an anti-asialo GM1 rabbit serum (Wako).

Interestingly, we could observe an considerable NK cells infiltration in tumor from mice immunized with P10s as illustrated in (**Figure 4a)** but not in the tumor from non-immunized animals (**Figure 4b)**. However, this infiltration did not correlate with the B cells infiltration as we could only observe **(Figure 5)** only a slight increase in the number of B cells infiltrating the tumor from immunized mice as compared to tumors in naïve animals. **In conclusion, the paucity and the localization of B cell infiltration makes it hard to relate the antitumor effect observed to an intratumoral interaction between the B cells and NK cells.**

A. **P10S +** B. **P10S -**

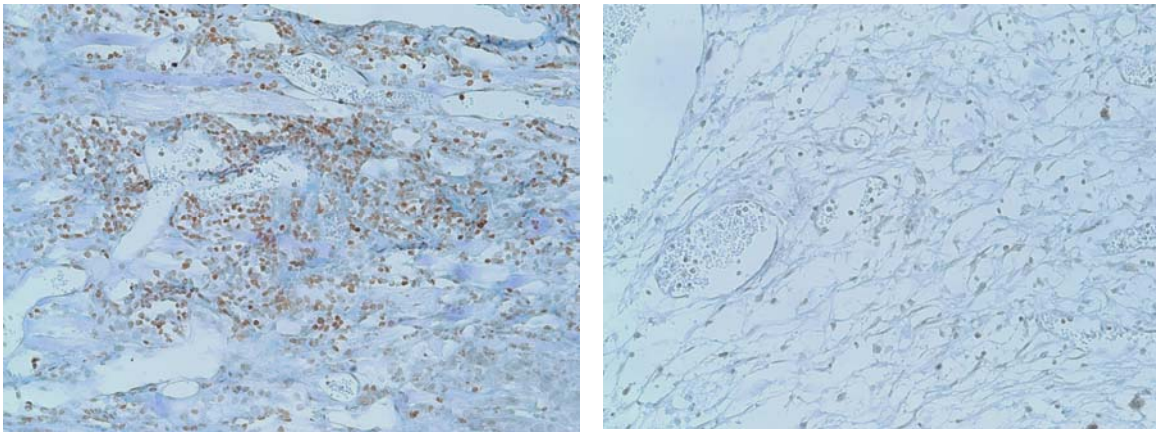
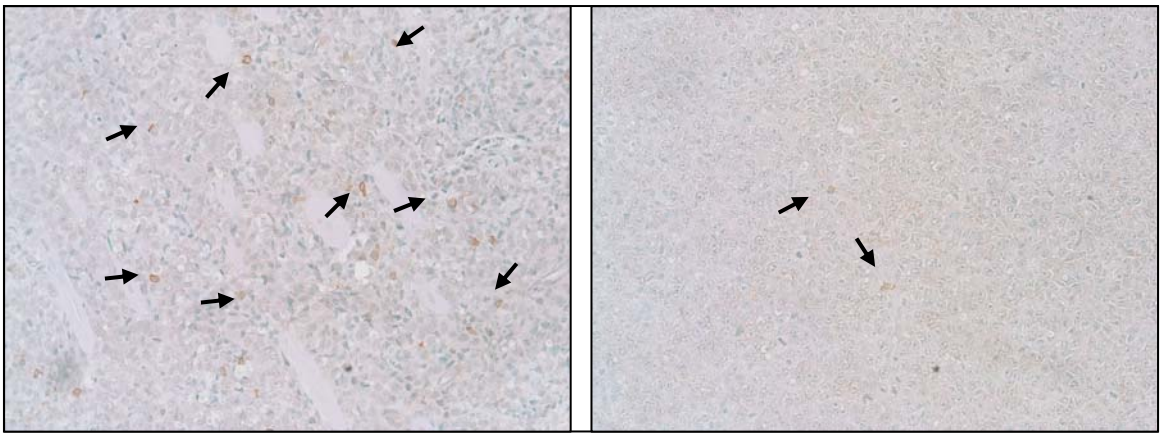


**Figure 4 NK cells infiltration in tumor mass after P10s immunization.** Tumor from mice immunized with P10s (Figure 4A) or non-immunized (Figure 4B) were excised 30 days after 4T1 cells inoculation. Sections were stained with an anti-asialoGM1

A. **P10S +** B. **P10S -**



C. **lymph node 4x** D. **lymph node 20x**

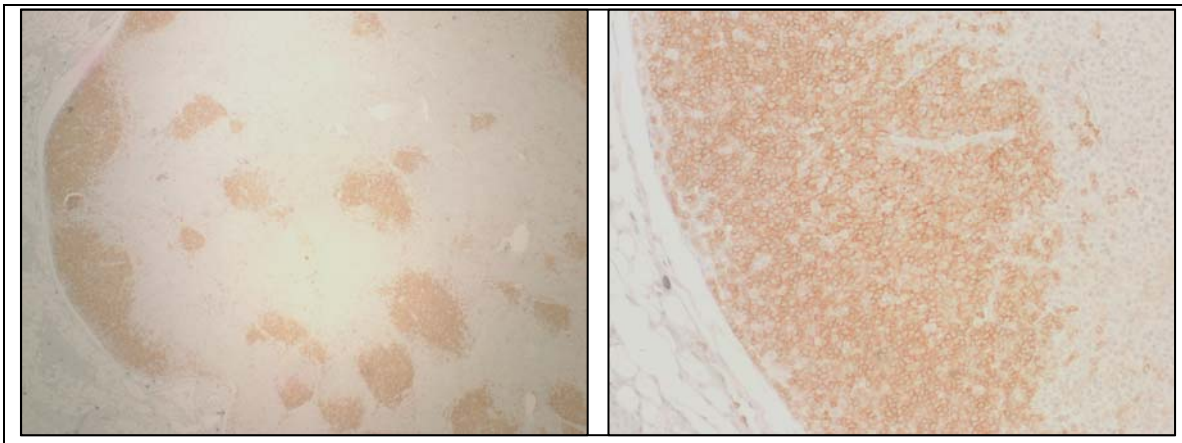


**Figure 5 B cells infiltration in Balb/c 4T1 tumor.** Tumor from mice immunized with P10S peptide (Figure 5A) or non-immunized (Figure 5B) were excised 30 days after 4T1 cells inoculation. Sections were stained with an anti-CD45R antibody (ebioscience; 5µg/ml). Sections from lymph node were used as positive control (Figure 5C&5D).

Although increases in B cell infiltrates in the tumor could not explain the activation of the NK cells, another possible local interaction dependent on P10s specific B cell activity is through the secreted IgM antibodies. We used an anti-mouse IgM antibody (Sigma) to detect the presence of IgM in the tumor of mice immunized with P10s. Although no deposition of immune complexes could be observed in the tumors of the immunized mice, the interstitial spaces stained diffusely for IgM (**Figure 6)**. This rare finding of extravascular IgM may be related to the leakiness of the tumor vessels and seems also related to the carbohydrate cross-reactive humoral immune response demonstrated in our previous report.

**These results suggest that the IgM antibodies elicited by P10s immunization reach and are able to interact with the tumor cells, promoting the tumoricidal function of the NK cells.**

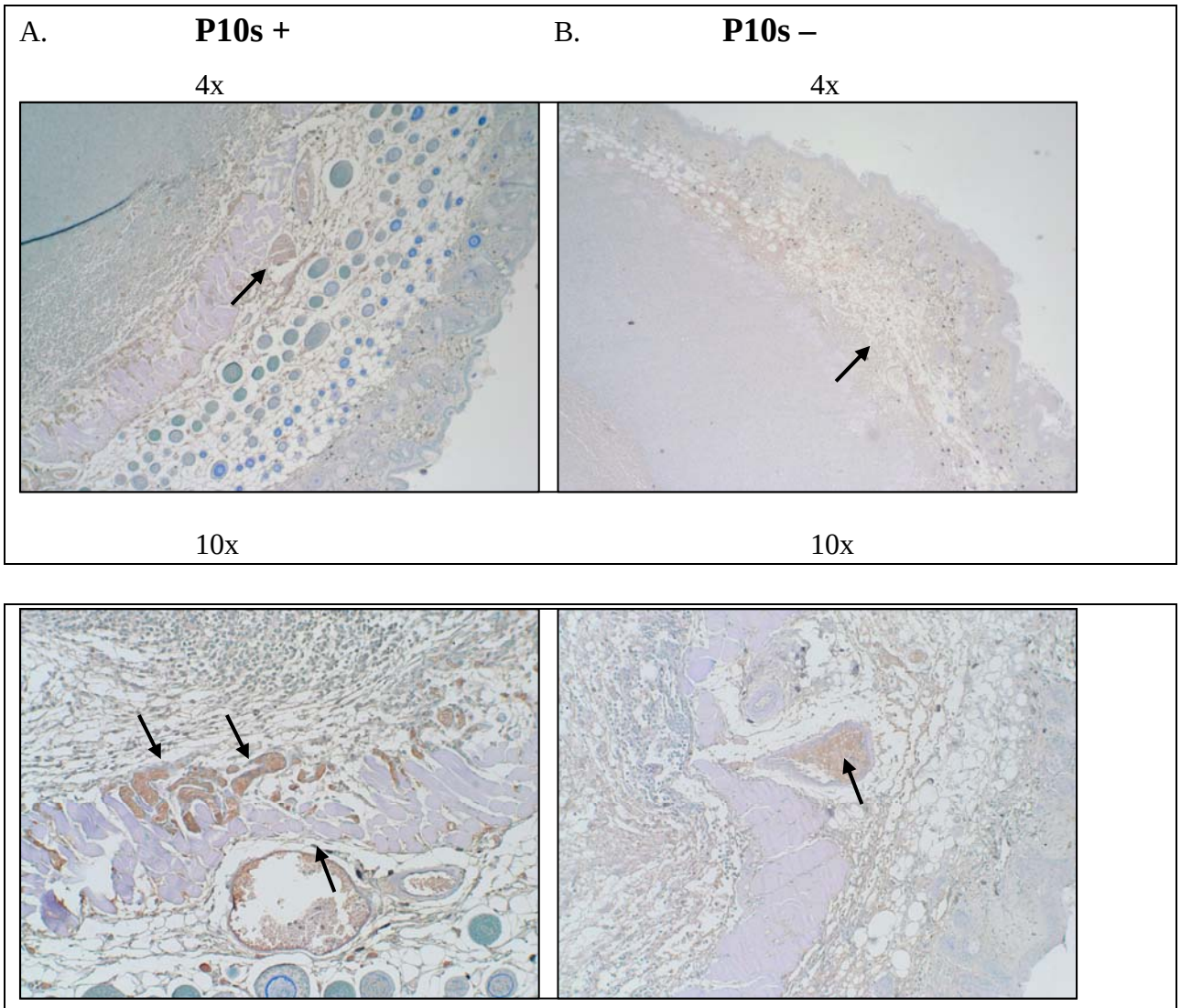



**Figure 6 IgM infiltration in tumor mass.** Tumor from mice immunized with P10s peptide (Figure 6A) or non-immunized (Figure 6B) were excised 21 days after MDA-231 cells inoculation. Sections were stained with an anti-mouse IgM antibody (sigma).

The mechanism by which IgMs activate or modulate NK cells activity is still unknown but several hypotheses are subject of an ongoing study. It is possible that the anti-P10s antibodies could neutralize carbohydrate binding to an inhibitory signal expressed on NK cells. It is known that murine NK cells express Sialic acid Ig like-lectin E (Siglec-E), which has the ability to bind disialogangliosides (5-7). . Indeed, engagement of siglec-E with its ligands is known to reduce the killing activity of NK cells (5). However we did not observe reduction of the NK cytotoxicity in the presence of Fab fragments of the anti-GD2/GD3 monoclonal antibody ME36.1 (data not shown). Another possible mechanism of action of IgM is through activating

the complement even when the lytic function of the latter is suppressed. It is also known that NK cells express the complement receptor 3 (aka CD11b). This type of antibody dependedent NK cytotoxicity is known as complement-dependent cell-mediated cytotoxicity. Involvement of this mechanism in the antitumor effect of p10s immunization is now being tested.

**To the best of our knowledge, this is the first report demonstrating that immunization with a CMP reduces tumor growth and improves tumor-bearing animal survival by modulating NK cells activity. In conclusion, we have shown in this 2007/2008 annual report that the CMP P10s presents numerous mimicry properties and that immunization with P10s peptide will induce antibodies capable of slowing down and improving survival of tumor bearing animals in a novel way.**

1.2. **Rabbit safety/toxicity study for P10s**.

In order to make sure that P10s immunization does not induce autoimmune pathology we performed a safety study in rabbits. The rabbit model has been used repeatedly to study neurological autoimmunity mediated by anti-ganglioside antibodies (e.g. Guillain-Barré syndrome, Miller-Fischer syndrome, etc.) because of the high expression of gangliosides in their peripheral nervous system **(8-11)**. . To this end we subcontracted this study with Biocon, Inc. (15801 Crabbs Branch Way, Rockville, MD 20855). Two rabbits were immunized subcutaneously with P10s and QS21 as adjuvant, every week for three weeks. Two non-immunized rabbits were used as negative controls. One week after the last immunization the animals were euthanized, necropsied and four organs were harvested (Brain, sciatic nerve, kidney and liver) for histopathology. In addition a complete urinalysis, complete CBC and differential was performed. Finally, we were provided with a Veterinary Pathologist report and Project Management study conduct report. (see appendix V).

**As mentioned in their report no negative clinical observations were made, which was consistent with our previous observation in mice, therefore, it is safe to conclude that P10s immunization does not induce Guillain-Barré Syndrome like-symptom in rabbits.** While not a GLP study this information will be passed along to the FDA when we present our GLP study.

| |
|---|
| Subtask 3: Complete animal safety/toxicity studies |
| Subtask 4: Prepare technical reports for in-house preclinical studies |

This is ongoing. P10s peptide has been synthesized and the certificate of analysis and verification letters are in appendix VI. The GLP study will commence on May 12$^{th}$ . More information will be given in the next annual report.

*Subtask 5: Prepare and submit pre-IND and IND documents to the FDA*

This is ongoing. We are developing the IND template and have written the IRB protocol (Appendix VII). We are working on the pre-IND and IND documents, we already gathered the information concerning the manufacturing company as well as the information concerning P10s.

**B. Aim2 Assure safety and determine Sufficient Immunogenic Dose (SID) of a mimotope vaccine in a Phase IA dose-escalation trial (year 2-3)**

*Subtask 1: Complete GMP manufacture, and obtain FDA and IRB approval prior to initiation Phase 1A*

A draft of the IRB protocol for Phase 1A is in its final stages of completion and will be review by our advisors. We have sent the draft of the IRB Protocol to Dr. Kimbark for further comments. As we were delayed due to the difficult solubility property of CMP 106 and that we had to change the CMP (P10s) we have reworked the SOW to reflect our time line for year 3. SOW for years 4-5 are the same as original application.

**Revised SOWs for years 3-5**

**YEAR 3**

| **Preclinical [Aim 1]** | |
|---|---|
| 1. Complete preclinical studies | Q2 |
| 2. Finalize in-house reports. | Q1-Q2 |
| 3. Request pre-IND meeting, Prepare pre-IND meeting package, Meet with FDA | Q3 |
| 4. File IND | Q4 |
| **Clinical [Aim 2 and 3]** | |
| 1. Finalize Investigator Brochure, protocols (Phase 1A and 1B), informed consent, etc | Q1-Q2 |
| 2. Obtain IRB and IBC approval from both UAMS and ARMY | Q2 |
| 3. Obtain FDA approval for IND | Q3 |
| 4. Initiate Phase IA trial | Q3 |
| 5. Develop validation assays for Immuno and Functional assays: ELISA | Q3-Q4 |
| 6. Calculate Coefficient Variance and modify procedures for validation | Q4 |
| 7. Complete Subject Enrollment, Clinical evaluations, Immunoassays, Biostatistical analyses | Q3-Q4 |
| 8. Implement data management system and clinical database | Q1-Q4 |

| | |
|---|---|
| 9. Complete data analyses for Phase IA study – if 9 patients required | Q4 |
| 10. Prepare Clinical Study Report for Phase IA | Q4 |

### YEAR 4

| | |
|---|---|
| **CMC - clinical supplies [Aim 3]** | |
| 1. Manufacture, Release more clinical supplies (if needed) for Phase 1B | Q1-Q2 |
| **Clinical – Phase IA [Aim 2]** | |
| 1. Complete data analyses for Phase IA study – latest if 18 patients required | Q2 |
| 2. Determine if SID has been met for repeat dose-finding for OID –latest if 18 patients required | Q2 |
| 3. Prepare Clinical Study Report for Phase IA – latest if 18 patients required | Q2 |
| **Clinical - Phase IB [Aim 3]** | |
| 1. Obtain FDA approval to initiate Phase IB | Q2 |
| 2. Initiate Phase IB trial | Q2 |
| 3. Enrollment of Study Subjects | Q2-Q3 |
| 4. Clinical Evaluation of Study Subjects while undergoing immunization | Q2-Q4 |
| 5. Standard clinical evaluation of Study Subjects after completion of immunization protocol | Q4 |
| 6. Perform antibody immunoassays | Q4 |
| 7. Perform laboratory studies of T cell responses | Q4 |

### YEAR 5

| | |
|---|---|
| **Clinical - Phase IB [Aim 3]** | |
| 1. Standard clinical evaluation of Study Subjects following immunization | Q1-Q2 |
| 2. Lock data base for Phase 1B clinical | Q2 |
| 3. Complete antibody data analyses | Q3 |
| 4. Complete analyses of T cell responses | Q3 |
| 5. Complete biostatistical analyses and prepare Summary Report | Q4 |

## KEY RESEARCH ACCOMPLISHMENTS

- **P10s immunization slows down tumor growth and increases survival of tumor-bearing animals by modulating NK cell activity.**

- **P10s immunization does not induce Guillain-Barre Syndrome like-symptom in rabbits.**

## REPORTABLE OUTCOMES

1) Results demonstrating that vaccination with P10s can enhance antibodies that work with NK cells to inhibit tumor growth to TACAs were presented as a poster at the recent American Association for Cancer Research meeting in San Diego and a manuscript is being prepared.

2) We are revising a manuscript entitled "Carbohydrate mimetic peptides induce tumor-associated carbohydrate-reactive antibodies in the absence of pathological autoimmunity". Leah Hennings, Cecile Artaud, Fariba Jousheghany, Behjatolah Monzavi-Karbassi, Anastas Pashov, Thomas Kieber-Emmons.

## CONCLUSION

In year two, we had experienced delays that delayed the initiation of our Phase I study by 6-7 months. We are aggressively pursing to end the GLP studies by October and setting up an IND meeting with the FDA for early December. We anticipate we shall be able to start our Phase IA studies in Feb 2009.

Within this year, we have finalized developing the infrastructure required to conduct the preclinical studies. The animal protocol (ACORP) was accepted and signed; the animal housing followed the rules and regulation of the 21CFR part 58; all Standard Operating Procedures are in place; all research staff is adequately trained. All the supply necessary for the study is available and ready to use.

The peptide P10s has been synthesized and a certificate of analysis provided. We have identified an unexpected and novel property of this carbohydrate mimetic peptide analog. This finding indicates that P10s will be a medical product with unique properties.

Rabbit immunization studies, while obtained in a research setting facility with research grade peptide, leads us to believe that no adverse immunopathology will be associated with GLP or GMP grade peptide mimotopes under the regulation of the 21CFR58 of the pre-clinical study.

References: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# Appendices

RESEARCH AND DEVELOPMENT COMMITTEE
CENTRAL ARKANSAS VETERANS
HEALTHCARE SYSTEM (CAVHS)
4300 West 7th Street
Little Rock, AR 72205

DATE: December 17, 2007

TITLE: "Determination of the Safety and Tolerability of Immunization With a LeY Peptide Mimotope Vaccine in Mice"

PRINCIPAL INVESTIGATOR: Kieber-Emmons, Thomas, M.D. (UAMS Slot 824)

1. Your research proposal as listed above was discussed by the R&D Committee at their last meeting on December 17, 2007.

2. This is your official APPROVAL letter.

3. By policy of the R&D Committee, if this study is funded by a private corporation or foundation and solely involves VA patients or resources, the funds must be administered at the Biomedical Research Foundation or CAVHS. If the study is funded by a private corporation or foundation and involves both UAMS and VA patients and resources, some portion of the funds should be administered at the Biomedical Research Foundation or CAVHS. Contact Loretta Phillips-Stillings at 257-4517 for further information and to work out details of the funding administration.

4. If your research has VA sensitive data involved (most particularly identifiable patient information or Category E animal studies), the data must be kept on VA network. If you have any questions this, please call the Research Administrative office at 257-4816.

LAWRENCE T. KIM, M.D.
Chairman

cc:
Loretta Stillings
Holly Rickman, Pharm.D.
Dana Pickett, IRB

UAMS
UNIVERSITY OF ARKANSAS
FOR MEDICAL SCIENCES

Department of Pathology, Breast Cancer Research Development
University of Arkansas for Medical Sciences
4301 W. Markham Street (slot 824)
Little Rock, AR 72205



# PROTOCOL 5-06-2 Determination of the safety and tolerability of immunization with LeY peptide mimotope vaccine in mice.

TABLE OF CONTENTS

## 1. Purpose

To determine the safety and tolerability of the administration of immunogenic mimetic peptides, which are designed to induce immune responses specific for tumor associated carbohydrate antigens (mimotopes), in mice.

## 2. Background

We have defined a series of carbohydrate mimetic peptides (mimotopes) that induce tumor-directed, carbohydrate-reactive humoral and cellular responses in experimental animals. Preclinical data demonstrate immunization with mimotopes reduces tumor burden and prolongs survival in mice. This safety and tolerability study in mice is a required step towards developing these molecules for clinical use as anti-tumor therapies for patients with breast cancer, with the ultimate goal of impacting relapse and prolonging survival.

## 3. Sponsor

University of Arkansas for Medical Sciences (UAMS)
4301 West Markham St. Slot 813
Little Rock, AR., 72205

## 4. Testing Facility and Key personnel

### a. Animal Facility

Veterinary Medical Unit (VMU)
Central Arkansas Veteran's Healthcare System
4300 West Seventh Street Research 151
Little Rock AR 72205 USA

### b. Histopathology Facility

UAMS - Arkansas Cancer Research Center-ACRC, Room 429
4301 West Markham St. Slot 725
Little Rock, AR., 72205

**c. Key personnel**

**Study Director**: Dr. Thomas Kieber-Emmons, PhD
**Veterinary Pathologist**: Dr. Leah Hennings, DVM

## 5. Identification and Test Control Substances

**a. Test Substance**

**Name:** P10S-PADRE
**Peptide Seq:** WRYTAPVHLGDG-aK-Cha-VAAWTLKAAa

Capitol letters – one letter code for L-amino acids
Small letters – one letter code for D-amino acids
Cha - Cyclohexylalanine

NeoMPS Inc (San Diego, CA 92126 · USA) will synthesize P10s mimotope covalently linked with PADRE.

PADRE is a synthetic, non-natural Pan HLA-DR binding peptide that binds with high or intermediate affinity to 15 of 16 of the most common HLA-DR types tested to date. Because of its binding promiscuity, PADRE should overcome the problems posed by the extreme polymorphism of HLA-DR molecules in the human population. Furthermore, the PADRE peptide was specifically engineered to be immunogenic in humans. This property represents another significant feature of PADRE, suggesting its potential utility as a carrier to induce T cell "help" in vaccine constructs designed for human use.

**b. Adjuvant**

QS-21 is an immunological adjuvant. Immunological adjuvants can modulate the humoral (i.e., stimulation of antibody quantity, avidity, affinity, persistence, and/or isotype switching) and/or cellular [(i.e., stimulation of delayed-type hypersensitivity and cytotoxic T lymphocytes (CTL)] immune responses to vaccine antigens. QS-21 has been shown to stimulate both humoral and cell-mediated immunity.

Source of QS-21:

Antigenics, Inc.
Corporate Office
630 Fifth Avenue Suite 2100
New York, New York 10111

QS-21 is a naturally occurring saponin molecule purified from the South American tree *Quillaja saponaria* Molina. It is a triterpene glycoside with the general structure of a quillaic acid 3, 28-O-bis glycoside with the formula $C_{92}H_{148}O_{46}$, and a molecular weight of 1990 Kd. QS-21 will be supplied in solid powder in an amber glass vial containing at least 100 mg or 1.0 gram of QS-21.

**c. Substance Storage**

- The P10s-PADRE will be received in powder condition and will be stored frozen at ≤-20° C for maximum stability until its use. Temperature logs will be maintained and recorded on business days (except holidays) from date of receipt. Logs will be treated as raw data.

- QS-21 will be stored at ≤ -20°C +/-5°C. The shelf life at this storage condition is four (4) years. The expiration date for QS-21 will be listed on the vial label and on the certificate of analysis. Temperature logs will be maintained and recorded on business days (except holidays) from date of stock solution preparation. Logs will be treated as raw data.

## 6. Test System

**a.** Test system characteristics

- Test system: Mouse
- Number of animals: 106 mice (10 extra mice are included in the event that some will not be acceptable)
- Body weight range: 15-25 grams
- Sex: Female

- Strain: Balb/c
- Age of the test system upon receipt: 4 to 6 weeks
- Source of supply:

Charles River Laboratories International, Inc.
Corporate Office
Attn: Research Models and Services
251 Ballardvale Street
Wilmington, MA 01887-1000

**b. Test System Justification**

The preferred animal model for toxicity testing is an animal expressing the relevant tumor antigen. The neolactoseries antigen LeY is not expressed in mice but a structurally related difucoganglioside, also mimicked by mimotope P10s, is endogenously expressed on murine tumors. Therefore, we propose a preclinical safety study to provide a gross characterization of the nature, frequency, and severity of adverse responses observed following vaccine administration in this tolerant mouse setting. The preclinical study will provide an initial basis upon which to determine the vaccine safety profile in a manner to support further study, including Phase 1 clinical testing.

**c. Group assignment/control of Bias**

Upon receiving, each animal will be assigned randomly to a cage. The randomization schedule for assignment to peptide treatment, sacrifice time, and urine-collection caging are shown in Tables A, B, and C (see Annex A, B and C), respectively, and were generated by a block randomization scheme implemented in Microsoft® Office Excel 2003. Block sizes were 4 for peptide P10s, 3 for sacrifice times, and 8 for urine-collection caging. Animals will be ear punched according to the group assignment tables (Table-A see Annex).

## 7. Experimental Design

**a. Formulation preparation**

Lab personnel will wear suitable protective clothing such as laboratory coat, and gloves according to UAMS policy.

The vaccine shall be prepared according to the Standard Operating Procedure IMM002. 20μg of QS-21 per mouse shall be mixed with the

appropriate quantity of peptide (100µg, 300µg and 500µg/mouse) in sterile phosphate buffered saline. The syringe will be loaded with the vaccine mixture in the animal procedure room just before its use.

**b. Immunization procedure using adjuvant / peptide mixture**

i) **Animals**

Female, 4-6 weeks old Balb/c mice will be purchased from Charles River Laboratories Inc. Upon arrival the mice will be quarantined for two weeks (Standard Operating Procedure ANCA007). The animals will be housed in the animal facility located at the Veterinary Medical Unit of the VA hospital. The animals receiving and care will be conducted according to Standard Operating Procedure ANCA003 and ANCA002 respectively.

Animals will be housed in group cages holding no more than 4 mice/cage according to Standard Operating Procedure ANCA002. The animals will be identified using an ear notching system and according to Standard Operating Procedure ANCA009. A log assigning animal notch number and cage number to their respective group will be maintained (see Annex A, Table-A).

There will be 3 test and 1 control group. 24 mice will be assigned per dosing group (3 time points with 8 mice sacrificed per time point).

The study groups are as follows:
**Adjuvant control:** 20µg QS-21 per mouse.
**Mimotope/adjuvant (Vaccine):** 100 µg, 300 µg, 500 µg per mouse
The total number of animals will be 96 mice.

**ii) Immunization Schedule and dosing**

Note: Immunization, weight measurement and observations should be done in the animal procedure room and follow the study calendar.

**Table 1.**

| Test article | EVENT | Doses Per mouse | STUDY WEEK | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13-18 | 19 | 20 | 21 |
| P10s-PADRE | Immunization | 500 ug | 24 | 24 | 16 | | | | 16 | | | | | | | 8 | | |
| | | 300 ug | 24 | 24 | 16 | | | | 16 | | | | | | | 8 | | |
| | | 100 ug | 24 | 24 | 16 | | | | 16 | | | | | | | 8 | | |
| | | adjuvant | 24 | 24 | 16 | | | | 16 | | | | | | | 8 | | |
| | Euthanasia | 500 ug | | | 8 | | | | | | 8 | | | | | | | 8 |
| | | 300 ug | | | 8 | | | | | | 8 | | | | | | | 8 |
| | | 100 ug | | | 8 | | | | | | 8 | | | | | | | 8 |
| | | adjuvant | | | 8 | | | | | | 8 | | | | | | | 8 |

96 animals will be used. Animals in each dose group will be injected subcutaneously with control or test article (P10s-PADRE) on weeks 1, 2, 3, 7, and 19 of the study. 20μg/mouse QS-21 in Saline buffer alone will be used as the control.

**Three doses of vaccine will be used:** 100 μg; 300 μg; 500 μg. All immunizations will be performed according to the Standard Operating Procedure IMM001.

After the immunizations are completed, syringes will be discarded in a biohazard disposal container. (Standard Operating Procedure SAF001).

## 8. In-Life Observations

### a. General health monitoring

All animals will be observed daily by experienced VMU animal care staff to assess their health and well-being. Visual inspection, as to general appearance of mice and condition of bedding, will be performed per Standard Operating Procedure ANCA002. The research assistant will specifically monitor the animals twice a week for injection site redness, swelling, heat, ulceration, or hair loss during two weeks following the immunization then weekly until the next injection. Any abnormality will be noted in the observation form for site injection reaction (see Annex H).

**b. Weight measurement**

Upon arrival at the animal care facility from the Charles River Laboratories Inc., mice will be weighed according to Standard Operating Procedure ANCA003. Animals will be weighed on a weekly basis thereafter on a calibrated scale per Standard Operating Procedure ANCA011. Weights will be recorded on the weighing form. (see Annex F)

**c. Morbidity**

Appropriate assessment techniques will include: evaluation of overall clinical condition including appearance, posture, body temperature, behavior and physiological responses; assessment of food and water intake; and weighing to determine changes in body weight. Animals that become moribund or lose greater than 10% of body weight over a 2 week period during the study will thereafter be euthanized and necropsied. Observation will be recorded on the gross pathology form. (see Annex K)

**d. Clinical Pathology**

Urine will be collected for three days in individual metabolic cages according to Standard Operating Procedure EQU007 and prior to scheduled necropsy for complete urinalysis. Five out of eight mice per group will be chosen randomly using a Microsoft® Office Excel 2003 Randomization spreadsheet for urinalysis testing. (see Annex C, Table-C).

The following parameters will be evaluated under GLP conditions at:

Rodent Clinical Pathology Core Laboratory
Central Arkansas Veterans Healthcare System
Research Services
4300 W. 7t$^{h}$ St.
Little Rock, AR 72205

| Appearance |
|---|
| Volume |
| Specific gravity |
| pH |
| Ketones |
| Bilirubin |
| Glucose |
| Occult blood |

| Urobilinogen |
|---|

Blood will be collected via cardiac puncture immediately postmortem, according to Standard Operating Procedure ANCA014.

The following parameters will be evaluated under GLP conditions at the Rodent Clinical Pathology Core Laboratory:

Central Arkansas Veterans Healthcare System
Research Services
4300 W. 7t$^{h}$ St.
Little Rock, AR 72205

| Leukocyte count, total and differential |
|---|
| Erythrocyte count |
| Hematocrit |
| Hemoglobin |
| Mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration (calculated) |
| Platelet count |

| Alkaline phosphatase |
|---|
| Aspartate Aminotransferase |
| Bilirubin, total |
| Calcium |
| Chloride |
| Creatinine |
| Gamma glutamyl transferase |
| Glucose |
| Lactate Dehydrogenase |
| Magnesium |
| Phosphorus |
| Potassium |
| Sodium |
| Total Protein |
| Urea Nitrogen |

**e. Necropsy**

On Week 3, prior to injection, and on weeks 9 and 21, two cages of 4 mice per group will be chosen according to the Microsoft® Office Excel 2003 randomization spreadsheet (see Annex B, Table-B) to be euthanized. Mice will be euthanized via an overdose of $CO_2$ (Standard Operating Procedure ANCA006) until movement and respiration cease. Death will be determined by lack of movement, and respiration. Animals will be necropsied according to Standard Operating Procedure ANCA013.

Necropsy should be performed upon sacrifice or unscheduled death with recording of organ weights and gross pathology and preservation of a complete list of tissues at necropsy under Standard Operating Procedure ANCA013.

Tissues evaluated for organ weight:

| | |
|---|---|
| Kidneys (paired) | Spleen |
| Liver | Heart |

See Annex for organ weighing form.

Tissues evaluated for gross lesions and preserved in 10% neutral formalin:

| | |
|---|---|
| Gross lesions | Mesenteric lymph nodes |
| | Ovaries |
| Brain and pituitary gland | Pancreas |
| Cecum | Rectum |
| Colon | Salivary glands, left and right Parotid, Sublingual, Submaxillary |
| Duodenum | |
| Esophagus | Skeletal muscle, quadriceps, left |
| Eyes, left and right | Skin, ventral and dorsal |
| Femur, left | Spinal cord in vertebral column |
| Injection site(s) | Spleen |
| Heart | Stomach |
| Ileum | Submandibular lymph nodes, left and right |
| Jejunum | Thymus |
| | Thyroids, left and right |
| Adrenal glands left and right | Tongue |
| Kidneys left and right | Trachea |
| Liver | Urinary Bladder |
| Lungs | Uterus |
| Mammary gland | Vagina |

**f. Tissue Processing**

Tissues will be collected and processed according to Standard Operating Procedure HIST004 and HIST006. All harvested organs will be embedded in paraffin blocks according to Standard Operating Procedure HIST003. Tissue from the control and high dose (500 µg) group will be sectioned according to Standard Operating Procedure HIST005 and then stained with hematoxylin and eosin (H&E) according to Standard Operating Procedure HIST001. Slides will be identified according to Standard Operating Procedure ANCA001 and examined by a veterinary pathologist. All gross lesions and target tissues will be evaluated in the mid- and low-dose groups.

## 9. Data analysis

**a. Data recording**

Appropriate entries in the experimental record should be made after each procedure and according to Standard Operating Procedure AM007 (See Annex F to K for forms used in the animal examination process)

**b. Protocol and Standard Operating Procedure Deviation**

All deviations to the Protocol or Standard Operating Procedure are to be reported immediately to the Study Director. All study staff are required to fill out a Protocol or Standard Operating Procedure Deviation form (see Annex D and E) if he/she deviates from the implemented Protocol or Standard Operating Procedures. All Protocol and Standard Operating Procedure Deviation forms are to be sent to the Study Director and the Administration Manager according to Standard Operating Procedure AM005.

**c. Evaluation of Test Results**

All gross lesions and all tissues from 8 mice in the highest dose group and per time point will be evaluated histologically and compared with all lesions and tissues from 8 adjuvant immunized control animals. If pathologies are noted in mice in the 500µg dose group compared to control animals, all tissues from the 300µg group will be evaluated. If pathologies are noted in this group, all tissues from the 100µg group will be evaluated. Animal weights and organ weights will be summarized within each group as medians and quartiles, and compared across groups using scatter plots in conjunction with Wilcoxon rank-sum tests. Abnormalities in blood, urine, wet tissue, and the histology slides will be

summarized by location as number and percent present in each group, and compared across groups using bar charts and/or profile.

**d. Reports**

A draft report will be provided to the Study Director for review. The final study report, including evaluation of the results, will be signed by the Study Director and veterinary pathologist and provided to the Sponsor.

**e. Records and Archives**

All raw data, records, protocol and report copies will be maintained according to standard operating procedure Standard Operating Procedure AM003.

**f. Regulatory requirement and good laboratory practices**

This study will be conducted in compliance with the U.S FDA Good Laboratory Practice Regulations (21CFR58) and according to standard operating procedures.

## 10. Confidentiality

All information regarding the identity of the test substance and data are considered to be confidential. No raw data, worksheets, data or information summaries, reports, or other information related to this study may be revealed or released to any third party without prior notification and authorization of the sponsor.

## 10. Protocol Approval Signatures

Study Director/Dr. Thomas Kieber-Emmons — May 7, 2008 Date

Management/Dr. Larry. D. Milne — 5/9/08 Date

Review:

# ANNEX

# A. Group Assignment: Table –A

| Mouse Order | Punch | Cage # | Mouse Order | Punch | Cage # | Mouse Order | Punch | Cage # |
|---|---|---|---|---|---|---|---|---|
| 1 | "0" | 300 µg, 1A | 37 | "L1" | 500 µg, 2A | 73 | "R1" | 500 µg, 3A |
| 2 | "0" | Control, 1A | 38 | "L1" | Control, 2A | 74 | "R1" | 300 µg, 3A |
| 3 | "0" | 100 µg, 1A | 39 | "L1" | 300 µg, 2A | 75 | "R1" | 100 µg, 3A |
| 4 | "0" | 500 µg, 1A | 40 | "L1" | 100 µg, 2A | 76 | "R1" | Control, 3A |
| 5 | "L1" | 300 µg, 1A | 41 | "R1" | 100 µg, 2A | 77 | "B1" | 500 µg, 3A |
| 6 | "L1" | Control, 1A | 42 | "R1" | Control, 2A | 78 | "B1" | 100 µg, 3A |
| 7 | "L1" | 100 µg, 1A | 43 | "R1" | 300 µg, 2A | 79 | "B1" | Control, 3A |
| 8 | "L1" | 500 µg, 1A | 44 | "R1" | 500 µg, 2A | 80 | "B1" | 300 µg, 3A |
| 9 | "R1" | 300 µg, 1A | 45 | "B1" | 500 µg, 2A | 81 | "0" | Control, 3B |
| 10 | "R1" | 500 µg, 1A | 46 | "B1" | 100 µg, 2A | 82 | "0" | 100 µg, 3B |
| 11 | "R1" | Control, 1A | 47 | "B1" | 300 µg, 2A | 83 | "0" | 300 µg, 3B |
| 12 | "R1" | 100 µg, 1A | 48 | "B1" | Control, 2A | 84 | "0" | 500 µg, 3B |
| 13 | "B1" | 500 µg, 1A | 49 | "0" | 300 µg, 2B | 85 | "L1" | Control l, 3B |
| 14 | "B1" | 300 µg, 1A | 50 | "0" | 500 µg, 2B | 86 | "L1" | 100 µg, 3B |
| 15 | "B1" | 100 µg, 1A | 51 | "0" | 100 µg, 2B | 87 | "L1" | 500 µg, 3B |
| 16 | "B1" | Control, 1A | 52 | "0" | Control, 2B | 88 | "L1" | 300 µg, 3B |
| 17 | "0" | 300 µg, 1B | 53 | "L1" | 100 µg, 2B | 89 | "R1" | 500 µg, 3B |
| 18 | "0" | 500 µg, 1B | 54 | "L1" | 500 µg, 2B | 90 | "R1" | Control, 3B |
| 19 | "0" | Control, 1B | 55 | "L1" | Control, 2B | 91 | "R1" | 100 µg, 3B |
| 20 | "0" | 100 µg, 1B | 56 | "L1" | 300 µg, 2B | 92 | "R1" | 300 µg, 3B |
| 21 | "L1" | Control, 1B | 57 | "R1" | Control, 2B | 93 | "B1" | 100 µg, 3B |
| 22 | "L1" | 100 µg, 1B | 58 | "R1" | 100 µg, 2B | 94 | "B1" | 300 µg, 3B |
| 23 | "L1" | 500 µg, 1B | 59 | "R1" | 300 µg, 2B | 95 | "B1" | 500 µg, 3B |
| 24 | "L1" | 300 µg, 1B | 60 | "R1" | 500 µg, 2B | 96 | "B1" | Control, 3B |
| 25 | "R1" | 500 µg, 1B | 61 | "B1" | 500 µg, 2B | | | |
| 26 | "R1" | 100 µg, 1B | 62 | "B1" | Control, 2B | | | |
| 27 | "R1" | Control, 1B | 63 | "B1" | 300 µg, 2B | | | |
| 28 | "R1" | 300 µg, 1B | 64 | "B1" | 100 µg, 2B | | | |
| 29 | "B1" | 100 µg, 1B | 65 | "0" | 300 µg, 3A | | | |
| 30 | "B1" | Control l, 1B | 66 | "0" | Control, 3A | | | |
| 31 | "B1" | 500 µg, 1B | 67 | "0" | 100 µg, 3A | | | |
| 32 | "B1" | 300 µg, 1B | 68 | "0" | 500 µg, 3A | | | |
| 33 | "0" | 100 µg, 2A | 69 | "L1" | 100 µg, 3A | | | |
| 34 | "0" | 500 µg, 2A | 70 | "L1" | 300 µg, 3A | | | |
| 35 | "0" | 300 µg, 2A | 71 | "L1" | 500 µg, 3A | | | |
| 36 | "0" | Control, 2A | 72 | "L1" | Control, 3A | | | |

## B. Animal Sacrifice order: Table-B

| Cage # | Sacrifice order | Cage # | Sacrifice order |
|---|---|---|---|
| 100-2A | 1 | 500-3A | 1 |
| 100-3B | 1 | 500-3B | 1 |
| 100-3A | 2 | 500-1A | 2 |
| 100-1B | 2 | 500-1B | 2 |
| 100-1A | 3 | 500-2A | 3 |
| 100-2B | 3 | 500-2B | 3 |
| | | | |
| 300-1A | 1 | Control-2A | 1 |
| 300-1B | 1 | Control-1B | 1 |
| 300-3A | 2 | Control-3A | 2 |
| 300-3B | 2 | Control-3B | 2 |
| 300-2A | 3 | Control-1A | 3 |
| 300-2B | 3 | Control-2B | 3 |

# C. Urinalysis assignment: Table-C

| Cage-punch | Assignment | Cage-punch | Assignment | Cage-punch | Assignment |
|---|---|---|---|---|---|
| 100-3A-O | 8 | 100-2A-O | 3 | 100-1A-O | 5 |
| 100-3A-L1 | 1 | 100-2A-L1 | 2 | 100-1A-L1 | 2 |
| 100-3A-R1 | 4 | 100-2A-R1 | 4 | 100-1A-R1 | 3 |
| 100-3A-B1 | 2 | 100-2A-B1 | 6 | 100-1A-B1 | 6 |
| 100-1B-O | 7 | 100-3B-O | 5 | 100-2B-O | 7 |
| 100-1B-L1 | 3 | 100-3B-L1 | 7 | 100-2B-L1 | 1 |
| 100-1B-R1 | 5 | 100-3B-R1 | 1 | 100-2B-R1 | 4 |
| 100-1B-B1 | 6 | 100-3B-B1 | 8 | 100-2B-B1 | 8 |
| | | | | | |
| 300-1A-O | 4 | 300-2A-O | 5 | 300-3A-O | 5 |
| 300-1A-L1 | 3 | 300-2A-L1 | 8 | 300-3A-L1 | 6 |
| 300-1A-R1 | 5 | 300-2A-R1 | 6 | 300-3A-R1 | 2 |
| 300-1A-B1 | 6 | 300-2A-B1 | 1 | 300-3A-B1 | 3 |
| 300-1B-O | 1 | 300-2B-O | 4 | 300-3B-O | 7 |
| 300-1B-L1 | 2 | 300-2B-L1 | 2 | 300-3B-L1 | 1 |
| 300-1B-R1 | 8 | 300-2B-R1 | 3 | 300-3B-R1 | 8 |
| 300-1B-B1 | 7 | 300-2B-B1 | 7 | 300-3B-B1 | 4 |
| | | | | | |
| 500-1A-O | 4 | 500-2A-O | 2 | 500-3A-O | 4 |
| 500-1A-L1 | 1 | 500-2A-L1 | 7 | 500-3A-L1 | 7 |
| 500-1A-R1 | 3 | 500-2A-R1 | 5 | 500-3A-R1 | 3 |
| 500-1A-B1 | 5 | 500-2A-B1 | 4 | 500-3A-B1 | 5 |
| 500-1B-O | 6 | 500-2B-O | 1 | 500-3B-O | 8 |
| 500-1B-L1 | 7 | 500-2B-L1 | 3 | 500-3B-L1 | 2 |
| 500-1B-R1 | 8 | 500-2B-R1 | 6 | 500-3B-R1 | 1 |
| 500-1B-B1 | 2 | 500-2B-B1 | 8 | 500-3B-B1 | 6 |
| | | | | | |
| Control-1A-O | 4 | Control-2A-O | 2 | Control-3A-O | 7 |
| Control-1A-L1 | 7 | Control-2A-L1 | 7 | Control-3A-L1 | 6 |
| Control-1A-R1 | 3 | Control-2A-R1 | 8 | Control-3A-R1 | 8 |
| Control-1A-B1 | 5 | Control-2A-B1 | 3 | Control-3A-B1 | 1 |
| Control-2B-O | 8 | Control-1B-O | 5 | Control-3B-O | 5 |
| Control-2B-L1 | 6 | Control-1B-L1 | 6 | Control-3B-L1 | 4 |
| Control-2B-R1 | 1 | Control-1B-R1 | 1 | Control-3B-R1 | 3 |
| Control-2B-B1 | 2 | Control-1B-B1 | 4 | Control-3B-B1 | 2 |

**Note:** Gray areas represent the mice that are selected for urinalysis

# D. Standard Operating Procedure (SOP) Deviation Form

| **Employee Information** | | | |
|---|---|---|---|
| Name: | | | |
| SAP Employee ID: | | Department: | |
| Job Title: | | Phone: | |
| Manager: | | | |
| SOP Number/ Title/ Version | | | |
| **SOP Deviation Details** | | | |
| **All deviations to a SOP are to be reported immediately.** Complete this form and give it to Dr. Thomas Kieber-Emmons in the Office of Breast Cancer Research in the Bio-Medical II building Room 306 at the University of Arkansas Medical Science (UAMS) or fax it to him at **501-526-5934**. | | | |
| **Who or What caused the deviation to the SOP?** | | | |
| **Please describe the SOP deviation.** | | | |
| **What effect did the deviation have on the study?** | | | |
| **What do you do to rectify the deviation?** | | | |
| **Who did you report the deviation to?** | | | |
| **How did you report the Deviation?** | | | |
| Signature: | | Date: | |
| | | | |

# E. Protocol Deviation Form

| **Employee Information** | | | |
|---|---|---|---|
| Employee Name: | | | |
| SAP Employee ID: | | Department: | |
| Job Title: | | Phone: | |
| Manager: | | | |
| Protocol Title and Version | | | |

| **Protocol Deviation Details** |
|---|
| **All deviations to the Protocol are to be reported immediately.** Complete this form and give it to Dr. Thomas Kieber-Emmons in the Office of Breast Cancer Research in the Bio-Medical II building Room 306 at the University of Arkansas Medical Science (UAMS) or fax it to him at **501-526-5934**. |
| **Who or What caused the deviation to the Protocol?** |
| **Please describe the protocol deviation.** |
| **What effect did the deviation have on the study?** |
| **What do you do to rectify the deviation?** |
| **Who did you report the deviation to?** |
| **How did you report the Deviation?** |

| | | | |
|---|---|---|---|
| Signature: | | Date: | |

# F. Weighing Form (measurement in g)

## Cage identification_____

| | Week | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mice ID | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| 0 | | | | | | | | | | | |
| L1 | | | | | | | | | | | |
| R1 | | | | | | | | | | | |
| B1 | | | | | | | | | | | |
| Initial & Date | | | | | | | | | | | |

| | Week | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mice ID | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | |
| 0 | | | | | | | | | | | |
| L1 | | | | | | | | | | | |
| R1 | | | | | | | | | | | |
| B1 | | | | | | | | | | | |
| Initial & Date | | | | | | | | | | | |

# G. Immunization Schedule Form

Cage identification_____

Test article Name: _____

Test article concentration: _____

| | Immunization #1 | | Immunization #2 | | Immunization #3 | | Immunization #4 | | Immunization #5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Mouse ID | Date | Initial | Date | Initial | Date | Initial | Date | Initial | Date | Initial |
| | | | | | | | | | | |
| L1 | | | | | | | | | | |
| R1 | | | | | | | | | | |
| B1 | | | | | | | | | | |

# H. Observation Form for Site Injection Reaction

**Group & Mouse identification**_____

Observation of redness (**1**), swelling (**2**), heat (**3**), ulceration (**4**), hair loss at the injection site (**5**) or Normal aspect (**N**)

Write the number corresponding to the observation or N for absence of site injection reaction.

| Week | Observation#1 / Date | Initial | Observation#2/ Date | Initial |
|---|---|---|---|---|
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |
| 6 | | | | |
| 7 | | | | |
| 8 | | | | |
| 9 | | | | |
| 10 | | | | |
| 11 | | | | |
| 12 | | | | |
| 13 | | | | |
| 14 | | | | |
| 15 | | | | |
| 16 | | | | |
| 17 | | | | |
| 18 | | | | |
| 19 | | | | |
| 20 | | | | |
| 21 | | | | |

Note: Black area means that only one observation of the injection site per week is needed

Pathologist Signature _____ Date _____

# I. Animal Sacrifice Schedule Form

**Cage identification**_____

| | Sacrifice | | | |
|---|---|---|---|---|
| Mouse ID | Date | Weight | Initial | Comment |
| O | | | | |
| L1 | | | | |
| R1 | | | | |
| B1 | | | | |

# J. Organ Weighing Form

**Cage identification** _____

Date_____ Time_____

| | Organ weighing (measurement in g) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mouse ID | Liver | Initial | Spleen | Initial | Kidney L/R | Initial | Heart | Initial |
| O | | | | | | | | |
| L1 | | | | | | | | |
| R1 | | | | | | | | |
| B1 | | | | | | | | |

UAMS
UNIVERSITY OF ARKANSAS FOR MEDICAL SCIENCES

Department of Pathology, Breast Cancer Research Development
University of Arkansas for Medical Sciences
4301 W. Markham Street (slot 824)
Little Rock, AR 72205



# K. Gross Pathology Report

**PI**_____ **IACUC#**_____

Animal identification_____

Prosector name _____ Date & Time_____

| Organs | Examined Y/N | Normal Y/N | Comments |
|---|---|---|---|
| Spleen | | | |
| Ovaries | | | |
| Uterus | | | |
| Urinary Bladder | | | |
| Vagina | | | |
| Rectum | | | |
| Colon Gross lesions | | | |
| Cecum | | | |
| Ileum | | | |
| Jejunum | | | |
| Duodenum | | | |
| Mesenteric lymph nodes | | | |
| Pancreas | | | |
| Stomach | | | |
| Left and Right adrenal glands | | | |
| Left and Right Kidneys | | | |
| Liver | | | |
| Salivary glands, left and right, Parotid, Sublingual, submaxillary | | | |
| Submandibular lymph nodes, left and right | | | |
| Tongue | | | |
| Trachea | | | |
| Thyroids, left and right | | | |
| Esophagus | | | |
| Thymus | | | |
| Lungs | | | |
| Heart | | | |
| Skin, ventral and dorsal | | | |
| Mammary gland | | | |
| Skeletal muscle, quadriceps, left | | | |
| Femur, left | | | |
| Eyes, left and Right | | | |
| Brain Gross lesions | | | |

**Prosector signature**_____
Pathologist date & signature_____

RUN DATE- 5/08/08 TIME- 12:47:29

BILLTO: 29450 - 03

VA MEDICAL CTR
ATTN: RM 6 B 116
151 LITTLE ROCK
4300 WEST 7TH
LITTLE ROCK AR

SHIPTO: 29450 - 012

VA HOSP
4300 WEST 7TH STREET
LITTLE ROCK AR

## ACKNOWLEDGEMENT

### ORDER SPECIFICATIONS

| | |
|---|---|
| CONTACT | HENNING |
| INVESTIGATOR | EMMONS |
| CRL CONTROL NO | 5469644 |
| P.O NO | VISA |
| RELEASE NO | |
| SPECIE | MICE |
| STRAIN | BALB/C |
| SEX | F |
| QUANTITY | 106 |
| WGT MIN | |
| WGT MAX | |
| AGE MIN | 35 |
| AGE MAX | 42 |
| PLANT | RALEIGH |
| AREA | R02 |
| START DATE | 5/09/08 |
| STOP DATE | 5/09/08 |
| SCHEDULED SHIP DATE | 5/09/08 |
| DELIVERY DATE | 5/12/08 |
| FREQUENCY | |
| AGE TYPE | A |
| DOB | CALC. DOB 03/31/08 |
| SPECIAL SERVICE | |
| DAYS PREGNANT | |
| COHORT DATE | |
| SURGERY-1 | |
| DAYS POST OP | |
| SURGERY-2 | |
| SP1 | |
| SP2 | |
| TEXT-1 | |
| TEXT-2 | |
| TEXT-3 | |
| NUMBER OF CRATES | 3 |

### PRICING

UNIT PRICE: 15.850

| | |
|---|---|
| EXTENDED ANIMAL PRICE | 1,680.10 |
| SURGERY-1 | .00 |
| SURGERY-2 | .00 |
| CRATES | .00 |
| OTHER | .00 |
| SURCHARGE | .00 |
| FREIGHT | .00 |
| SALES TAX | .00 |
| TOTAL AMOUNT | 1,680.10 |

### * * * COMMENTS * * *

Speak to your Customer Service Representative about receiving these Charles River Laboratories Order Status/Acknowledgement reports via email.

Clinical Pathology Report
4/25/08
Leah Hennings, DVM

I have examined the clinical pathology results from the 4-rabbit preliminary, non-GLP study performed by Anti-Lytics, INC.

Minor deviancies in electrolytes, trygliceride, and erythrocytic parameters from the reported historical controls were consistent for both groups of animals, and are judged to be non-significant findings.

Rabbits in group 1 had elevated creatinine phosphokinase. Creatine phosphokinase is released from damaged muscle.Although I am blinded to the treatment groups, I suspect the elevation to be due to the injection of the peptide plus adjuvant into the muscle. This would be expected to incite more muscle damage than injection of control saline.

I find no sign of significant adverse reaction to the vaccination in these clinical pathology data.

Leah Hennings, DVM

4/25/08

CONFIDENTIAL-DRAFT

March 31, 2008

Cecile Artaud
University of Arkansas for Medical Sciences
Professor of Pathology
Josetta Wilkins Chair in Breast Cancer Research
Director Breast Cancer Research Development
Email: artaud.cecile@uams.edu

Dear Dr. Artaud:

Thank you for your interest in NeoMPS, Inc. (formerly known as Multiple Peptide Systems).

I have enclosed the following documents related to the project at hand:

1) Proposal for the FDA CMC section inquiries.
2) The quotation including additional tests per FDA request

Please let me know if I may be of any further assistance.

Sincerely,

Robert A. Hagopian
Director, Business Development

# SUMMARY PROVIDED IN SUPPORT OF THE MANUFACTURE OF THE DRUG SUBSTANCE

**Manufacturing facilities:**

Manufacturing of the peptide (drug substance) will be performed at NeoMPS Inc. in facilities licensed by the California Department of Health Services, Food and Drug Division (Drug / Device manufacturing license # 63358) and registered with the Food and Drug Administration (FDA registration # 2028155).

## I. PEPTIDE DESCRIPTION:

The sequence of the peptide (drug substance) to be manufactured for University of Arkansas for Medical Sciences is listed below using the single-letter code with the corresponding molecular weights, empirical formula and associated counter ion.

| Peptide Sequence | Molecular Weight | Empirical formula | Counter ion | Peptide Content (Calculated) | Acetate Content (Calculated) |
|---|---|---|---|---|---|
| | | | | | |
| H-WRYTAPVHLGDG-aK-Cha-VAAWTLKAAa-$NH_2$ | 2706.2 amu. | $C_{128}H_{197}N_{35}O_{30}$ | Acetate | 90 % | 10 % |

**Peptide Characterization QC testing and Specifications:**

| | QC Testing | Method | Specification |
|---|---|---|---|
| | | | |
| 1 | Appearance | Visual Observation | White to off-white powder |
| 2 | Identity | Mass Spectral Analysis | Correct MW ± 1 amu. |
| 3 | Sequencing | MS/MS for sequence confirmation | Correct Sequence |
| 4 | Identity | Amino Acid Analysis (AAA) for Identity | Correct Composition |
| 5 | Net peptide content (NPC) | Quantitative AAA for peptide content | ≥75 % |
| 6 | Purity | RP-HPLC USP <621> | ≥ 95% purity |
| 7 | Water Content | Karl Fischer, USP <921> | ≤15 % |
| 8 | Residual solvents | Gas Chromatography, USP <467> | Report results |
| 9 | Quantification of Counter Ion, acetate | RP-HPLC, USP <621> | ≤15 % |
| 10 | Total Fluorine | Combustion / Ion Selective Electrode USP <471> | <0.5% |
| 11 | Bacterial Endotoxin | Kinetic chromogenic, USP <85> | Report results |
| 12 | Specific rotation | Polarimetry, USP <781> | Report result |

Notes: Purity: Spontaneous pyroglutamic acid formation for peptides starting with glutamic acid or glutamine residues is not considered an impurity. Spontaneous cysteine and methionine oxidation for peptides that have these residues is not considered an impurity.
Tests 1-6 are performed in-house per applicable SOP's
Tests 7-12 may be subcontracted per applicable SOP (K0001)
See Appendix I for sample Certificate of Analysis of an exemplary product manufactured at NeoMPS for similar application

## II. SYNTHESIS / METHOD OF MANUFACTURE:

Chain assembly will be performed by solid phase peptide synthesis using Fmoc chemistry starting with the appropriate Fmoc Rink resin[2].

### A. Starting materials:

1. Amino acids and derivatives:

   The protected amino acid used for the synthesis of these peptides will be of non-animal origin. The following natural amino acid derivatives, obtained from qualified vendors, will be used, as applicable, for the manufacture of the peptides: The amino acids will be received tested and released per applicable standard operating procedure (SOP).

| **Amino acid derivative** | **Approved supplier (selected)** |
|---|---|
| | |
| Fmoc-Alanine | Senn Chemicals, Nova Biochem |
| Fmoc-D-Alanine | Senn Chemicals, Nova Biochem |
| Fmoc-Aspartic acid (tertiobutyl) | Senn Chemicals, Nova Biochem |
| Fmoc- $N^{\gamma}$ 2,2,4,6,7 pentamethyldihydrobenzofuran-5-sulfonyl-Arginine | Senn Chemicals, Nova Biochem |
| Fmoc-Cyclohexylalanine | Senn Chemicals, Nova Biochem |
| Fmoc-Glycine | Senn Chemicals, Nova Biochem |
| Fmoc-Histidine(trityl) | Senn Chemicals, Nova Biochem |
| Fmoc Isoleucine | Senn Chemicals, Nova Biochem |
| Fmoc Leucine | Senn Chemicals, Nova Biochem |
| Fmoc-$N^{\varepsilon}$-Benzyloxycarbonyl-Lysine | Senn Chemicals, Nova Biochem |
| Fmoc-Proline | Senn Chemicals, Nova Biochem |
| Fmoc-O-tertiobutyl-Threonine | Senn Chemicals, Nova Biochem |
| Fmoc-Tryptophan(benzyloxycarbonyl) | Senn Chemicals, Nova Biochem |
| Fmoc-O-tertiobutyl-Tyrosine | Senn Chemicals, Nova Biochem |

[1]Fmoc: fluorenylmethyloxycarbonyl. Name of the temporary protecting group of the N-α-amino group of the protected amino acids used during the synthesis. Also used to characterize the type of chemistry used for the peptide chain assembly.

[2]Merrifield, R.B. 1963. Solid phase peptide synthesis I: The synthesis of a tetrapeptide. J. Am. Chem. Soc. 85: 2149-2004.

2. Resins Used for Peptide Synthesis:

   The following Fmoc derivatized amino acid resin, obtained from qualified vendors, were used for the manufacture of the peptide:

| **Resin** | **Selected Approved Supplier** |
|---|---|
| | |
| Fmoc Rink Resin | Senn Chemicals, Nova Biochem |
| | |

3. Other Chemicals

| Chemical Name | Abbrev. | Grade | Selected Suppliers | Step Used in Manufacture | Minimum Acceptable Purity |
|---|---|---|---|---|---|
| 2-Propanol | IPA | HPLC | EMD Chemical | Synthesis | 99.5% |
| Acetic Acid | HOAc | ACS | Fisher/VWR | Cleavage / Purification | 99.5% |
| Acetonitrile | ACN | HPLC | EMD Chemical | Purification | 99.5% |
| Argon | Ar | Industrial | West Air | Packaging | 99.99% |
| Diisopropylcarbodiimide | DIC | N/A | Albatross | Synthesis | 94% |
| Diisopropylethylamine | DIEA | N/A | Albatross | Synthesis | 98% |
| Dimethylformamide | DMF | ACS | EMD Chemical | Synthesis | 99.5% |
| Hydroxybenzotriazole | HOBt | $99^{+}$% | Albatross | Synthesis | 95% |
| Methanol | MeOH | HPLC | EMD Chemical | Column Cleaning | 99.5% |
| Methylbenzhydrylamine resin | N/A | N/A | Senn Chemicals | Synthesis | N/A |
| Methylene Chloride | DCM | ACS | EMD Chemical | Synthesis | 99.5% |
| Nitrogen | $N_2$ | NF | West Air | Synthesis | 99.99% |
| Piperidine | | N/A | ChemImpex/Matrix | Synthesis | 98% |
| Trifluoroacetic Acid | TFA | Biograde | Halocarbon | Cleavage / Purification | 98% |
| tert-Butyl Methyl Ether | MTBE | HPLC | Fisher | Cleavage | 99% |
| Water | $H_2O$ | USP Purified | NeoMPS | All, except Synthesis | NLT 15 MΩ |

**B. Flow Chart of Synthesis Cycle:**

Figure 1 below describes the anticipated cycle for solid phase "Fmoc" chemistry of the peptide H-WRYTAPVHLGDG-aK-Cha-VAAWTLKAAa-$NH_2$. The starting resin is Fmoc Rink resin. The cycle is repeated for adding the remainder amino acids in the sequence listed below.

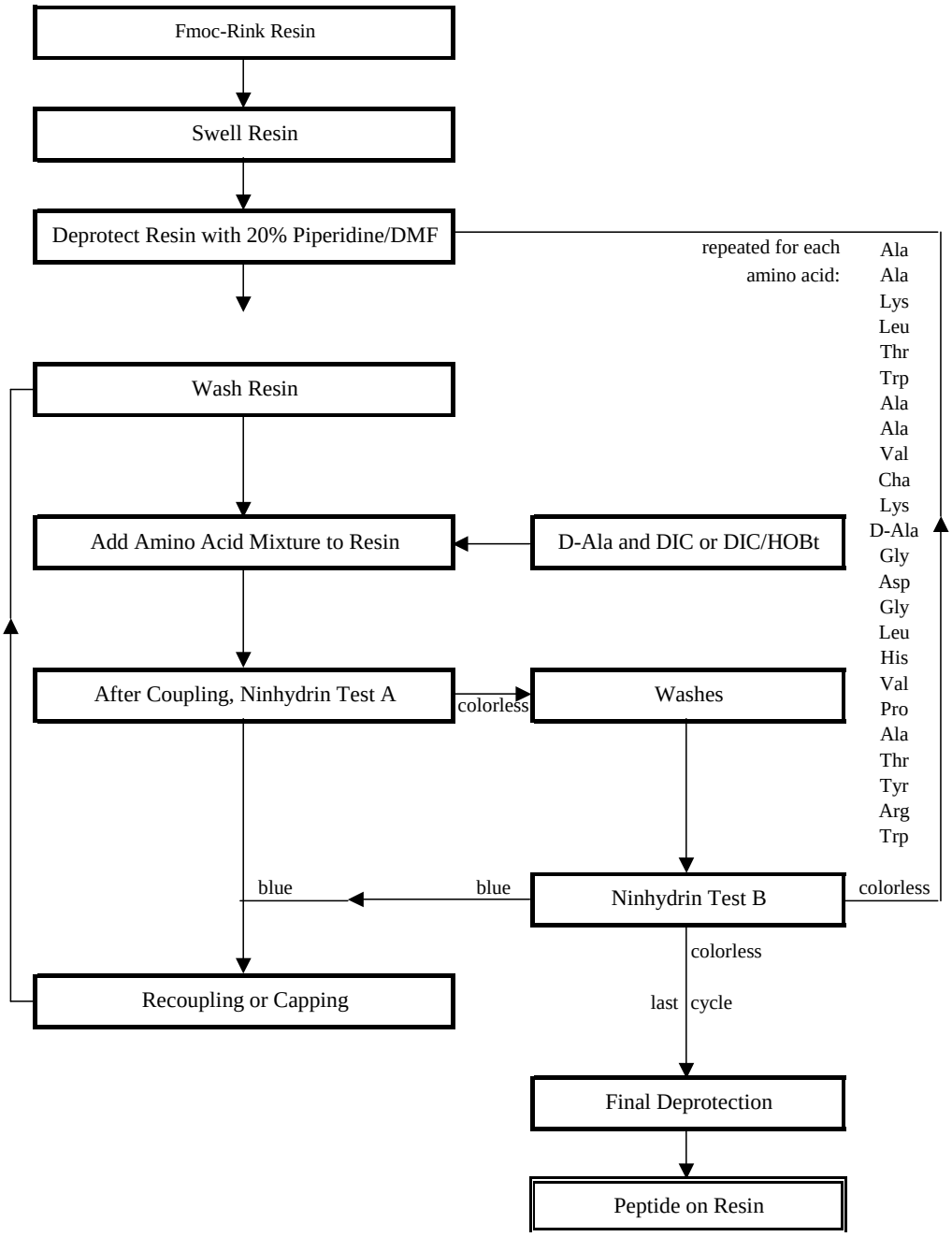


*Figure 1, anticipated coupling cycle for solid phase peptide synthesis.*

**C. Detailed Description of Synthesis:**

The general process of preparation of a peptide by solid phase synthesis can be broken down into 6 elemental steps:

1) Synthesis or assembly of the peptide on the resin; 2) side chain deprotection and cleavage of the peptide from the resin; 3) single or multiple step purification; 4) counter anion exchange, if needed; 5) packaging and labeling; and 6) QC testing and release.

1. Synthesis:

   The procedure used for the preparation of the peptide on resin is the general procedure described in the original paper of Merrifield[1] in 1963, with minor modifications.

   Synthesis will be performed for the peptide at room temperature, in an appropriate reaction vessel equipped with a fritted filter at the bottom for easy solvent wash and filtration of the solid support. Synthesis will be performed at an appropriate scale on the appropriate FmocRink resin[2]. Fmoc chemistry will be used throughout the peptide chain assembly. Calculation of solvent wash volumes, with the exception of the deprotection step, will be based on 8 ml/g of starting resin. Deprotection steps with 20% piperidine in dimethylformamide (DMF) will be performed with 12 ml per gram of starting resin, as a precaution to ensure complete Fmoc removal. Refer to page 5 for coupling cycle.

   The extent of coupling of each amino acid will be monitored by the ninhydrin test[3] and recoupling will be performed if a positive ninhydrin test result is obtained.

   After completion of the last coupling step (first amino acid in the peptide sequence), the resin will be treated with 20% piperidine/DMF to remove the N-terminal α-amino Fmoc protecting group.

   The deprotected resin will be washed with DCM and IPA, dried overnight under a nitrogen stream in the reaction vessel. The final yield of dry peptide resin will be determined and compared to the theoretically calculated amount as an in process check.

2. Cleavage of peptide:

   Each peptide will be cleaved from the resin using trifluoroacetic acid (TFA) and a number of appropriate cation scavengers to minimize side reactions. The cleaved peptide will be extracted in TFA and precipitated in ether. The precipitate will be filtered and dried under vacuum.

   In process checks includes a comparison of the crude yield versus theoretical yield, purity check by RP-HPLC and mass analysis for identity confirmation.

[1]Merrifield, R.B. 1963. Solid phase peptide synthesis I: The synthesis of a tetrapeptide. J. Am. Chem. Soc. 85: 2149-2004.

[2]Fmoc: fluorenylmethyloxycarbonyl. Name of the temporary protecting group of the N-α-amino group of the protected amino acids used during the synthesis. Also used to characterize the type of chemistry used for the peptide chain assembly.

[3]Sarin, V.K., et al. 1981. Quantitative monitoring of solid-phase peptide synthesis by the ninhydrin reaction. Anal. Biochemistry. 177: 147-157.

3. Purification:

   The crude peptide obtained from the cleavage procedure will be purified using a "Waters" preparative HPLC system, using reverse phase chromatography.

   A $C_{18}$ derivatized silica column will serve as the stationary phase. Column size will be 5 cm in diameter and 30 cm in length. The mobile phase will be buffered water and acetonitrile. The gradient used will be tailored to achieve the purity specification. Care will be taken to clean the column after each purification run, as demonstrated with achievement of a flat baseline following methanol and acetonitrile wash of the column.

   A single step purification should be sufficient to achieve the desired purity, although a second purification step may be required to achieve the required minimal purity specification by analytical HPLC. The column separation performance will be assessed with the analysis of the collected samples described below.

   Analysis of the collected samples from purification will be accomplished using an analytical Beckman HPLC system and a Vydac (or equivalent) $C_{18}$ 4.6 mm x 250 mm, 5 μm, 300 Å analytical column. The fractions containing pure peptide will be pooled and lyophilized to dryness in preparation for the exchange step or the second purification step. Lyophilization will be performed on a Virtis 25EL lyophilizer.

   The purification step may yield the peptide as the proper counter ion in which case the exchange step to the final counter ion may not be necessary.

4. Ion Exchange Chromatography:

   If necessary, ion exchange will be performed on an ion exchange resin, to convert the peptide to the acetate counter ion.

   Lyophilization of the peptide to dryness will be performed on a Virtis 25EL lyophilizer.

5. Packaging and labeling:

   Packaging of the peptide will be performed under argon in polyethylene containers with polypropylene closures. Alternatively glass amber vials with teflon lining with plastic closures.

6. QC testing / Release:

   Product will be released for distribution after the analytical testing (see page 2) is completed, reviewed and reported on the Certificate of Analysis (see attached example Appendix I).

## III. FLOW CHART OF MANUFACTURING:

The anticipated flow chart of manufacturing should be similar to the sample outlined below *(figure 2)*, assuming a single step purification followed by a counter ion exchange from the trifluoroacetate to the acetate salt.

As indicated in the manufacturing details, a two-step purification may be adopted in order to achieve the desired purity level. In this case the ion exchange step may not be necessary.

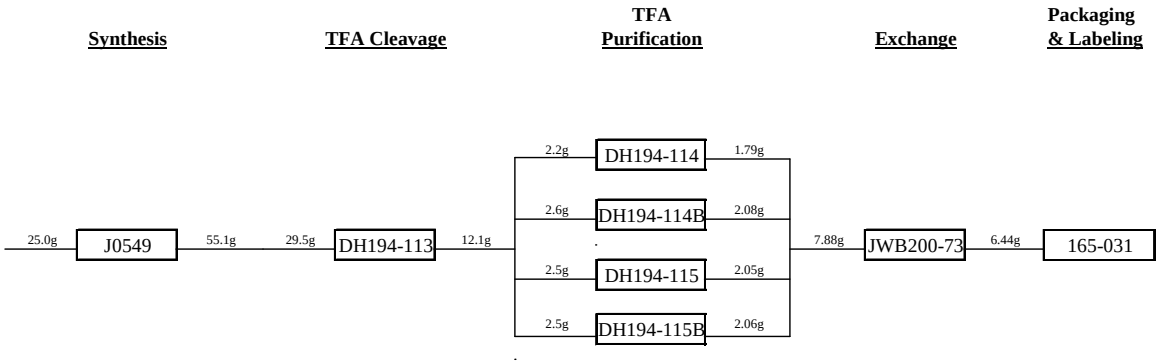


*Figure 2, Sample flow chart of manufacture.*

## IV. PROCESS CONTROLS:

### Summary of In-Process Controls:

| Process Step | Parameter | Acceptance Criteria |
|---|---|---|
| Synthesis | Ninhydrin test | Yellow color |
| | Final weight of peptide-resin | 85 - 110 % of theoretical weight |
| TFA Cleavage | Theoretical Yield | State result |
| | Purity check by HPLC | For information only |
| | Mass Spectral analysis | Confirm identity |
| Purification | Purity check by HPLC | > 95 % |
| Pooling/Exchange (If necessary) | Purity check by HPLC | > 95 % |
| | Final weight of peptide acetate | 85 - 100% of theoretical weight |

## V. REFERENCE STANDARD:

Approximately 500-1000 milligrams of the peptide manufactured in this campaign will be retained for the purpose of maintaining a reference standard.

## VI. CONTAINERS AND CLOSURES:

Packaging of the peptide will be performed under argon in polyethylene containers with polypropylene closures. Alternatively glass amber vials with Teflon lining with plastic closures.

## VII. PRODUCT RELEASE:

Product will be released for distribution after the analytical testing (see page 2) is completed, reviewed and reported on the Certificate of Analysis (see attached example Appendix I).

**APPENDIX I**
**Sample Certificate of Analysis**

NeoMPS, Inc.
PolyPeptide Laboratories Group

PolyPeptide Laboratories

# *CERTIFICATE OF ANALYSIS*

Lot Number: **XF207 // 042-31-12** Grade: **Research Grade**

Name: **P10S-C-PADRE** Quantity: **200 mg**

Sequence: **H-WRYTAPVHLGDG-aK-Cha-VAAWTLKAAa-NH2**

Counter Ion: **Acetate**

| TEST | METHOD | SPECIFICATIONS | RESULTS |
|---|---|---|---|
| Appearance | Visual Observation | White to off-white powder | White powder |
| Identity | Mass Spectrometry (MS) | M.W. (average) = 2706.2<br>M.W. (monoisotopic) = 2704.5<br>± 1 amu | $(M+H2)^{2+}/2$ = 1353.6<br>$(M+H3)^{3+}/3$ = 903.0<br>After deconvolution:<br>M = 2705.6 |
| | Amino Acid Analysis (AAA) | Asx 1 Tyr 1<br>His 1 Val 2<br>Gly 2 Leu 2<br>Thr 2 Lys 2<br>Cha 1 Pro 1<br>Ala 7 Trp 2<br>Arg 1<br>Report Results | Asx 1.0 Tyr 1.0<br>His 1.0 Val 2.0<br>Gly 2.1 Leu 2.1<br>Thr 1.7 Lys 2.0<br>Cha ND* Pro 1.0<br>Ala 7.2 Trp ND*<br>Arg 1.0<br>*Not determined |
| Purity Assay | RP-HPLC (Area normalized) | ≥ 95% | 96.3% |
| Acetate Content (HOAc) | Ion Chromatography with UV detection | Report Results | 5% |
| Net Peptide Content (NPC) | Based on %N | Report Results | 87% |
| C, H, N | C = 56.81%<br>H = 7.34%<br>N = 18.12% | Report Results | C = 52.20%<br>H = 7.34%<br>N = 15.74% |



9395 Cabot Drive, San Diego, California 92126 Tel: (858) 408-0808 or (800) 338-4965
Fax: (858) 408-0799 or (800) 654-5592 website: www.neomps.com email: mps@mps-sd.com

NeoMPS, Inc.
PolyPeptide Laboratories Group

PolyPeptide Laboratories

# *CERTIFICATE OF ANALYSIS*

Lot Number: **XF207 // 042-31-12** Grade: **Research Grade**

Name: **P10S-C-PADRE** Quantity: **200 mg**

Sequence: **H-WRYTAPVHLGDG-aK-Cha-VAAWTLKAAa-NH2**

Counter Ion: **Acetate**

| TEST | METHOD | SPECIFICATIONS | RESULTS |
|---|---|---|---|
| Bacterial Endotoxin | Gel Clot USP <85> | Report Results | < 0.41 EU/mg |
| Water Content ($H_2O$) | Karl Fischer USP <921> | Report Results | 6% |
| Total Mass Balance | Calculation NPC + HOAc + $H_2O$ | Report Results | 98% |

Date of Manufacture: **June 14, 2007**
Re-test Date: **June 14, 2008**

Site of Manufacture: **San Diego, CA, USA**

Quality Assurance by _____ Date 4/16/08



9395 Cabot Drive, San Diego, California 92126 Tel: (858) 408-0808 or (800) 338-4965
Fax: (858) 408-0799 or (800) 654-5592 website: www.neomps.com email: mps@mps-sd.com

# *NeoMPS*

System : Beckman Nouveau
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Acquired : Feb 22, 2008 09:05:19

Channel A Method

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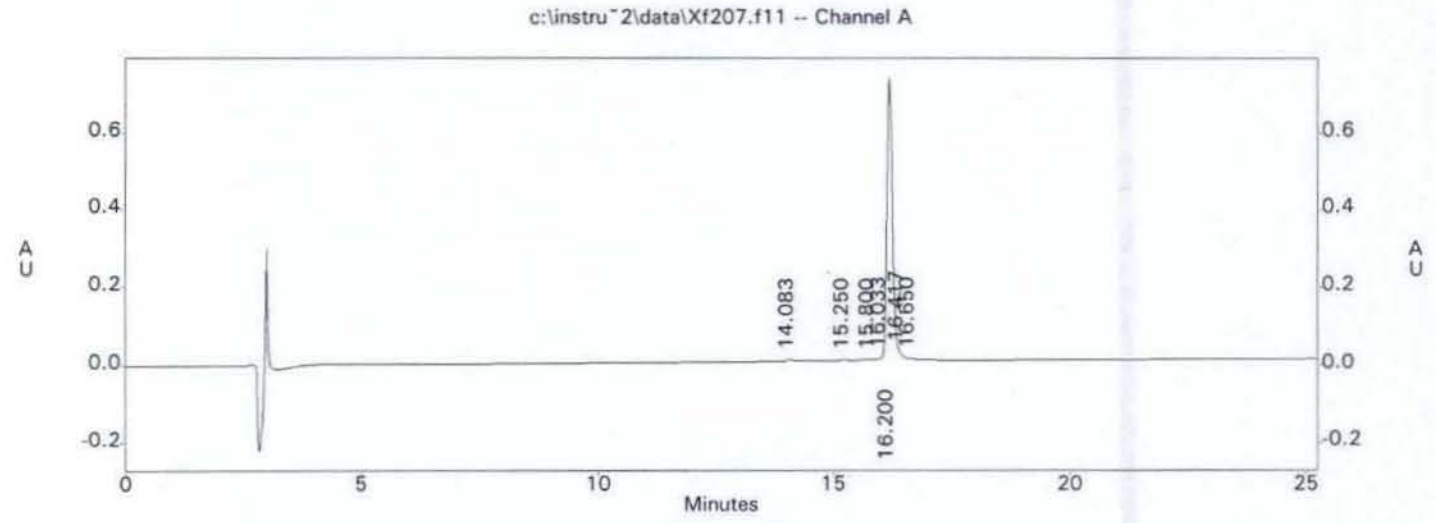


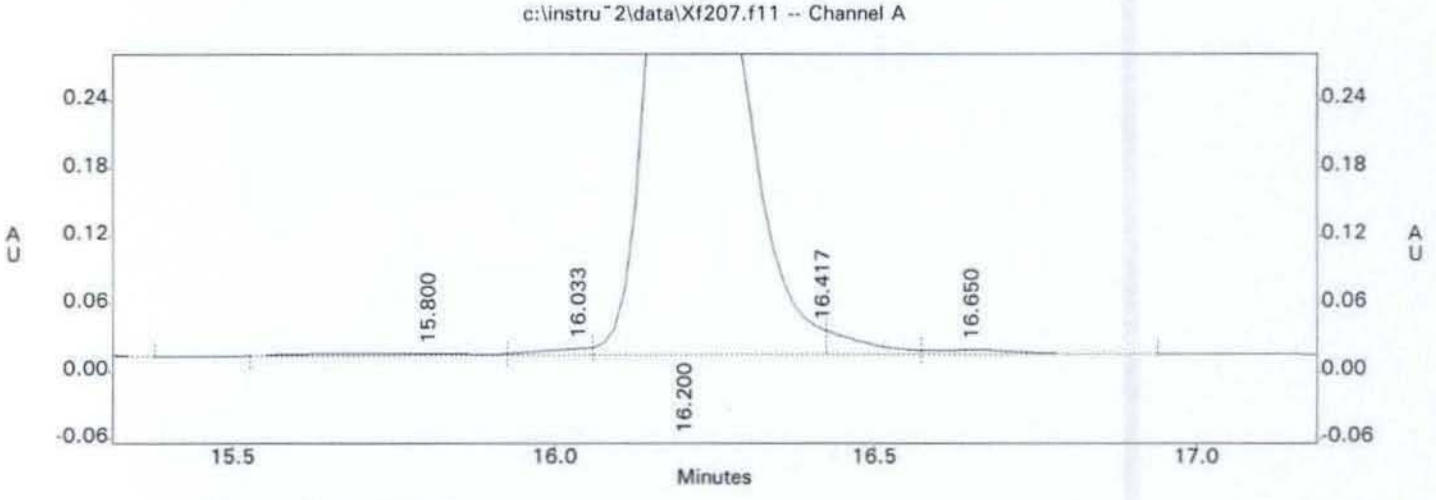


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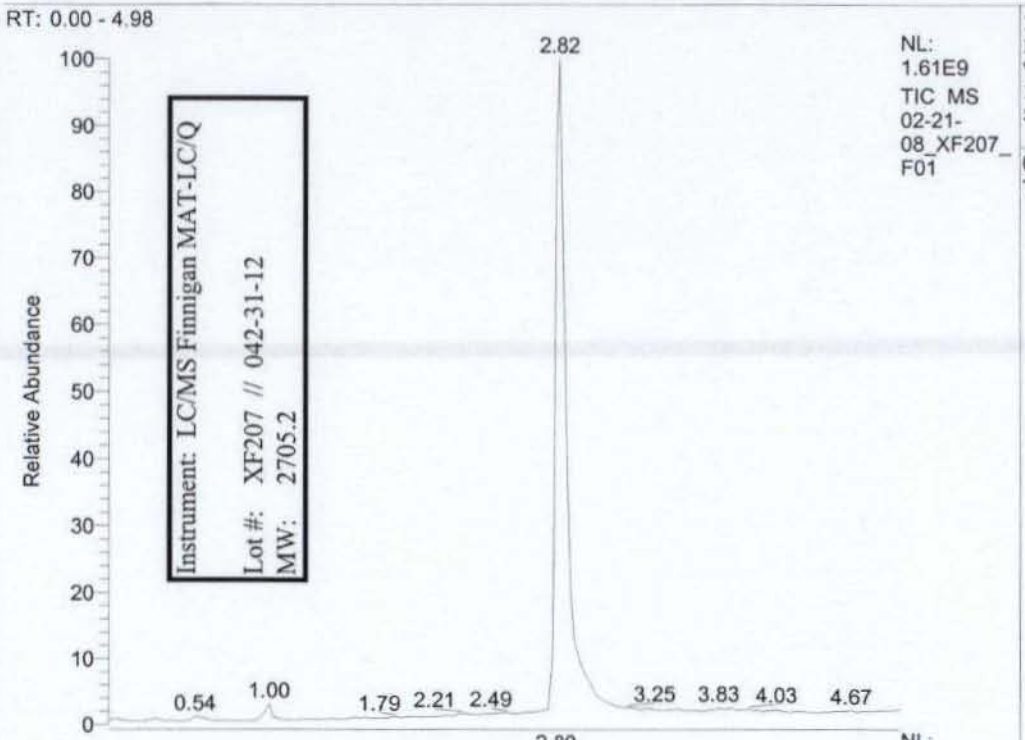


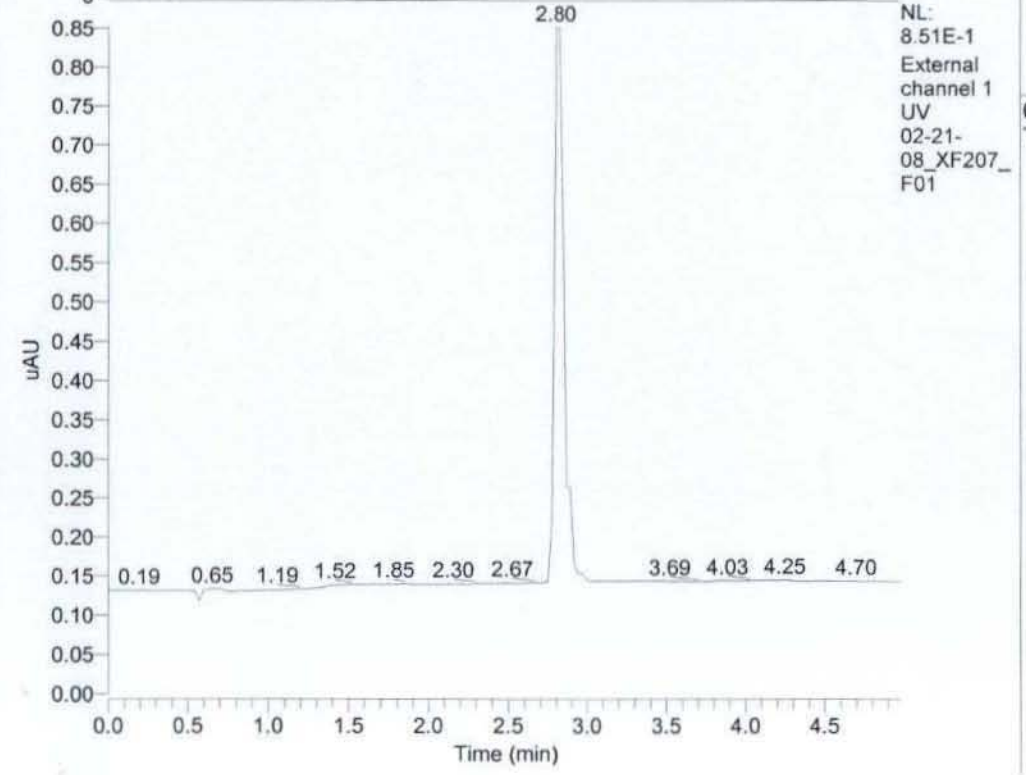


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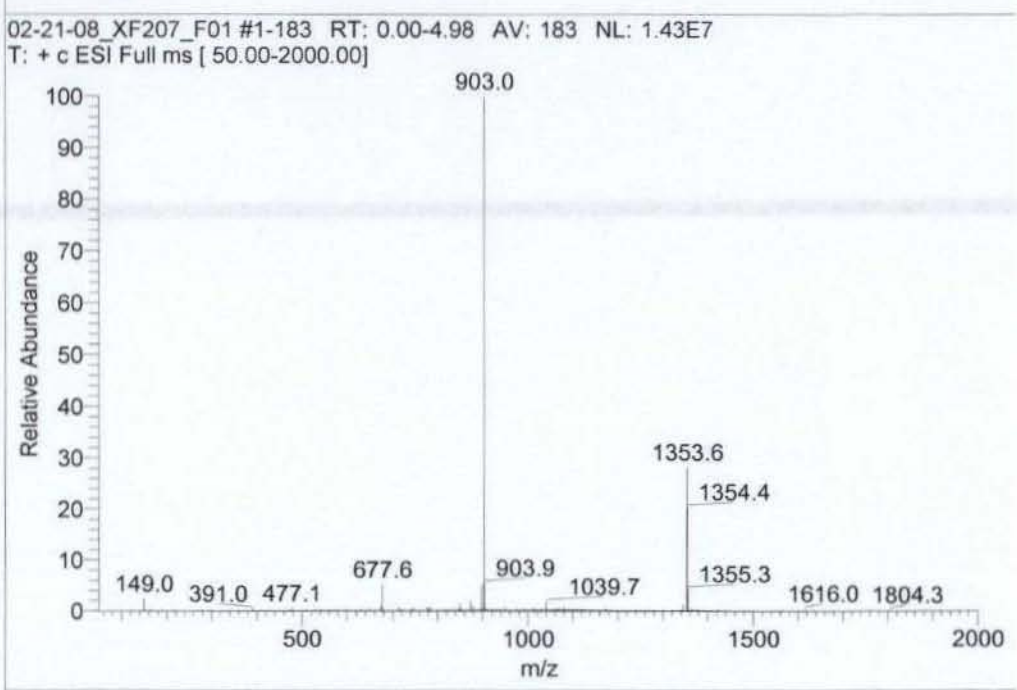


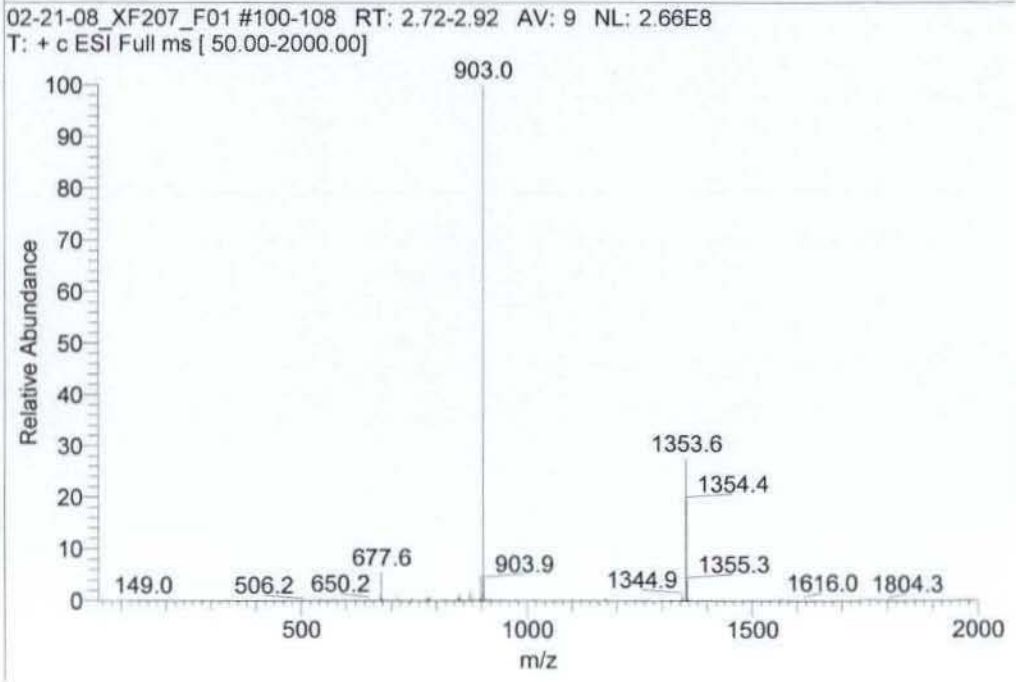

# Protocol 1

# In Support of Proposal Titled:

# "Vaccination of High Risk Breast Cancer Patients"

# Phase 1 Dose-Finding Study of a Carbohydrate Mimotope-conjugate vaccine with QS-21

**Thomas Kieber-Emmons, Ph.D., Principal Investigator**
**Laura Hutchins, M.D., Co- Principal Investigator**
**Issam Makhoul, M.D., Subinvestigator**
**Eric Siegel, MS, Biostatistician**

# Table of Contents

## 1.0 OBJECTIVES

### 1.1 Primary Objectives

1) Determine the safety and tolerability of a peptide mimotope-based vaccine upon immunization of breast cancer patients.

2) Determine whether immunization with the vaccine generates a humoral response against the Lewis Y (LeY) antigen to define a Sufficient Immunogenic Dose (SID) of the peptide mimotope-based vaccine in a Phase I dose-escalation trial.

### 1.2. Secondary Objectives.

Determine DTH responses to the immunizing mimotope

### 1.3 Drugs to be used.

Mimotope P10s-PADRE
QS-21

### 1.4 Endpoints of interest

#### 1.4.1 Primary Endpoints:

**Occurrence of Dose Limiting Toxicity (DLT):** Patients will be evaluated for toxicity using the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0. (http://ctep.cancer.gov). A toxicity of Grade 3 or higher will be considered a DLT if it is deemed to be related to the vaccine or any of its components.

**Adequacy of Immune Response:** To evaluate the adequacy of immune responses, serum will be collected following the time points on the study calendar (section: 9.0 ). IgM and IgG titers to LeY will be evaluated by ELISA. Titer is defined as the highest serum dilution yielding an $OD_{405} \geq 0.15$, in accordance with previous studies (1). A positive LeY-directed immune response will be defined as an anti-LeY serum antibody titer of 1:40 for a baseline pre-vaccination titer of 0 or a ≥4-fold increase for a baseline titer >0 (10). Patients will be judged to have had an adequate immune response if they have a positive LeY-directed immune response at any one of the five designated time points shown on the study calendar (section: 9.0).

#### 1.4.2 Secondary Endpoint:

**Delayed Type Hypersensitivity (DTH) Response:** To evaluate DTH responses, as secondary to the sc injected vaccines, study subjects will be skin-tested against immunizing mimotope. 100 µg of mimotope P10s-PADRE will be injected intradermally (id) on the patient's back. Tetanus- Diptheria Toxoid Antigen will be used as a control antigen, administered id at a separate location on the patients back. DTH response will be defined as the amount of induration surrounding the injection site, and will be measured using calipers and reported in mm across the longest and shortest diameters at 48 hrs.

## 2.0 BACKGROUND

### Anticipated anti-cancer impact of carbohydrate-targeted vaccines

The potential impact of vaccines that induce responses to tumor-associated carbohydrate antigens (TACA) is demonstrated by clinical trials where patient survival significantly correlates with carbohydrate-reactive IgM levels (2). Such results suggest that TACA-targeting vaccines might have a beneficial effect on the course of malignant disease. TACA-induced responses could augment naturally-occurring carbohydrate-reactive IgM antibodies that trigger apoptosis of tumor cells (3). TACA are attractive targets because the majority of cell-surface proteins and lipids are glycosylated and the glycosyl moiety is fundamental to the biological functions of these molecules in cancer cells (4,5). A unique advantage in targeting TACA is that multiple proteins and lipids on the cancer cell can be modified with the same carbohydrate structure. Thus targeting the carbohydrate antigen broadens the spectrum of antigens recognized by the immune response thereby lowering the risk of developing resistant tumors due to the loss of any one antigen (6). In addition, antibodies that recognize glycolipids are more apt to mediate complement-dependent cytotoxicity (CDC) and may, therefore, be more cytotoxic to tumor cells than antibodies that recognize protein antigens (7). Furthermore, preclinical studies support the hypothesis that vaccine-induced responses against TACA might have their greatest impact in the adjuvant setting as such responses inhibit tumor outgrowth in metastatic models (8,9).

### Approaches to augment immune responses to TACA

A variety of approaches are being taken to generate responses to TACA. Because TACA are T-cell–independent antigens and self-antigens, conjugation to immunologic carrier protein is perceived essential to recruit T cell help in antibody generation. Conjugation does not, however, assure an increase in immunogenicity because conjugation strategies do not uniformly enhance carbohydrate immunogenicity (10,11). Furthermore, even with conjugation, the lack of induction of cellular immune responses that would amplify TACA-reactive humoral responses necessitates constant boosting with vaccine. Representative examples of carbohydrate-based conjugate vaccines in clinical development include those directed toward gangliosides (12-14), polysialic acid (15), Globo-H (16), Lewis Y (LeY) (1), and the STn antigen (17).

An approach predicted to facilitate cellular responses exploits the molecular mimicry of TACA by protein surrogates, as they are T-cell–dependent antigens. Clinical characterizations of anti-idiotypic antibodies that mimic the GD3 ganglioside antigen (18) and GD2 (19) have been described. Peptide mimics of TACA (peptide mimotopes) are alternatives to anti-idiotypic antibodies. The characterization of peptide mimotopes is at present limited to preclinical studies. Peptide mimotopes have been described for GD2 (20-22), GD3 (23), sialylated Lewis a/x (24), and LeY (20, 25). Importantly, in preclinical prophylactic and therapeutic vaccination studies, peptide mimotopes were efficacious in eliciting immune responses that reduced tumor burden and inhibited metastatic outgrowth (8, 25, 26). Thus, peptide mimotopes of TACA represent a new and very promising tool to overcome T-cell independence and to increase the efficiency of the immune response to glycan antigens.

**Benefits of peptide mimotope immunization**

There are several benefits to vaccination strategies that employ peptide mimotopes of TACA. First, peptide mimotopes function as xenoantigens and, consequently, provide an advantage to overcome tolerance to carbohydrate self-antigens. Antibodies induced by peptide mimotopes are thought to have low affinities for TACA; specific targeting of tumor cells is due in part to over-expression of the carbohydrate antigen on tumor cells, which compensates for the low affinity of the carbohydrate cross-reactive antibodies (27). In addition, mimotope-induced antibodies preferentially recognize the terminal residues of the TACA oligosaccharides, which are often structurally distinct from those found on normal cells (28). Thus potential immunopathology due to destruction of normal tissue is minimized.

Second, peptide mimotopes have the potential to overcome immune deficiencies that suppress vaccine-induced carbohydrate-directed responses (29). Unlike carbohydrate antigens and carbohydrate-conjugate vaccines, peptide mimotopes also prime B- and T-cells for subsequent memory of carbohydrate antigens, facilitating long-term surveillance through recall of carbohydrate immune responses (30). This effect may minimize the need for constant boosting. In addition, they can functionally emulate conserved structures of TACA, inducing antibodies that recognize multiple TACA, and therefore functioning like a TACA multivalent vaccine (29).

Third, peptide mimotopes can be manipulated in ways that TACA cannot. Peptide mimotopes can be engineered to induce CD8+ T cells cross-reactive with tumor-associated glycopeptides and/or to induce CD4+ T cells that benefit the further expansion of CD8+ T cells and B-cells (26, 31 ). The ability to induce a humoral carbohydrate cross-reactive response, a CD4+ T helper (Th) response, and a CD8+ cytotoxic T lymphocyte (CTL) response with one simple inoculation is a novel approach to vaccination. Therefore, peptide mimotopes hold the potential to generate a multifaceted TACA-reactive immune response.

**Target carbohydrate antigens expressed on breast cancer cells**

Tumors expressing high levels of certain types of TACA exhibit greater metastasis than those expressing low levels of these antigens, which negatively impacts prognosis (32-34). In breast cancer, the LeY, STn, KH-1, and globo H carbohydrate antigens are considered prime vaccine candidates because of their tissue distribution (35, 36). In

particular, LeY has long been recognized as a potential target for immunotherapy because it is expressed in 70–90% of tumors of epithelial origin (37). LeY-reactive serum and monoclonal antibodies to LeY mediate CDC (1, 25) and antibody-dependent cellular cytotoxicity ADCC (1, 37) of human breast cancer cell lines. Immunotherapy targeting LeY might also provide a complementary, potentially synergistic approach to those targeting erbB protein, as anti-LeY antibody recognition of LeY-modified erbB affects signaling via these receptors (38). At present LeY-conjugate vaccines appear to have only a limited ability to induce anti-LeY immune responses in humans (1). Our *in vitro* studies demonstrate that peptide mimotopes of LeY and structurally-related gangliosides induce serum antibodies in mice that recognize the appropriate carbohydrate antigens on human or murine breast cancer cell lines (25, 39). Our *in vivo* studies demonstrate that the peptide mimotopes induce sustained immunity to these antigens (8, 25, 26). Collectively, these data provide the experimental foundation for evaluating peptide mimotopes as potential cancer vaccines in patients with breast cancer.

## 3.0 DRUG INFORMATION

**See the investigator's Brochure for complete information.**

### 3.1a QS-21 Complete information is available in the Invetigator's Brochure: Appendix ?

**aa. Description**

QS-21 is a naturally occurring saponin molecule purified from the South American tree Quillaja saponaria Molina. It is a triterpene glycoside with the general structure of a quillaic acid 3, 28-O-bis glycoside with the formula $C_{92}H_{148}O_{46}$, and a molecular weight of 1990 Kd.

The QS-21 (generic name) molecule consists of a mixture of two structural isomers that cannot be separated easily by reversed-phase HPLC:

QS-21-V1: 3-O-β-D-galactopyranosyl-(1→2) -[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl-quillaic acid 28-O- β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl-(1→2)-3-[5-O-α-L-arabinofuranosyl 3,5-dihydroxy-6methyl-octanoyl]-3,5-di-hydroxy-6-methyl-octanoyl]- β-D-fucopyranoside. [CAS 141256-04-4]

QS-21-V2: 3-O-β-D-galactopyranosyl-(1→2) -[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl-quillaic acid 28-O-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl-(1→2)-3-[5-O-α-L-arabinofuranosyl 3,5-dihydroxy-6methyl-octanoyl]-3,5-di-hydroxy-6-methyl-octanoyl]-β-D-fucopyranoside. [CAS 14563352-9].

QS-21 consists of two structural isomers designated QS-21-V1 and QS-21-V2.

These have identical chemical formulas and molecular weights and differ in one terminal pentose. V1 and V2 are present in the product at a typical ratio of V1:V2 of approximately 2:1. Both isomers have biological activity over a similar dose range for adjuvant function[1], and are believed to be the active ingredient in the product.

**ba. <u>Mechanism of action and toxicology</u>**

QS-21 is an immunological adjuvant that has been shown to stimulate both humoral and cell-mediated immunity.

Antibody Quantity and Isotypes

QS-21 has been shown to stimulate antibody responses to various vaccine antigens in mice, guinea pigs, rats, rhesus monkeys, and baboons. The IgG response to a QS-21adjuvanted antigen is typically increased 10- to 1000-fold compared to that induced by unadjuvanted antigen. Whereas 5-20 µg of QS-21 has adjuvant activity in mice, doses of 50 to 100 µg are effective in nonhuman primates.

Functional Antibody Responses

QS-21 improves functional antibody responses (viral neutralizing and bactericidal antibody) in animals. QS-21 was shown to stimulate a substantially higher serum neutralizing antibody titer to HIV-1 after immunization of baboons with HIV glycoprotein gp120 in comparison to the viral neutralizing response raised by gp120/aluminum hydroxide.

Antigen Dose-Sparing

Two biweekly SC doses of 5 µg of ovalbumin, adjuvanted with 10 µg QS-21, induced titers in C57BL/6 mice that were over 100-fold higher than the titers induced by 125 µg of unadjuvanted ovalbumin. A similar dose-sparing effect was noted with HIV-1 gp120 in guinea pigs[11] and in baboons. These findings suggest that QS-21 adjuvant could be used to decrease the minimum immunogenic dose of antigen (antigen dose-sparing).

Persistence of Response/Immunological Memory

Adjuvants have also been shown to affect the duration of the antibody response.

This was evaluated with various HIV-1 gp120 formulations with or without QS-21 in guinea pigs. After a single immunization, a peak serum antibody titer was observed, followed by a pseudo first-order decay of antibody. This was followed by a plateau of low level antibody titers that were approximately 10-fold lower than the peak. This plateau of low level antibody after the decay phase is called antibody persistence. Various antigen formulations, including QS-21, MF-59, aluminum hydroxide, and no adjuvant, yielded similar antibody decay kinetics. However, considerable differences were observed between adjuvants for peak titers and plateau titers. A strong correlation between peak titers and plateau titers was observed. Of formulations tested, QS-21 induced the highest peak titers and the highest plateau titers.

Modulation of Cell-Mediated Immune Response

QS-21 has been shown to induce $CD8^+$ cytotoxic T-lymphocyte (CTL) responses to subunit antigens in mice. Induction of antigen-specific $CD8^+$ CTL is believed to play a critical role for protection against and clearance of infection with intracellular organisms such as viruses or some bacteria and parasites. Other examples of QS-21 induction of CTLs to subunit antigens in mice include HIV-1 gp160D, rec-CMV gB, and RSV fusion protein. The levels of CTLs induced by RSV fusion protein/QS-21 were comparable to those achieved by experimental infection with RSV. SIV envelope and gag vaccines formulated with QS-21 have also induced CTLs in rhesus monkeys.

**Pharmacokinetics**

Not applicable. Biodistribution and pharmacokinetic studies of vaccine components (antigen and adjuvant) are not typically conducted in vaccine studies. Pharmacokinetic studies of QS-21 in humans have not been carried out.

**c. <u>Storage and stability</u>**

QS-21 is soluble in aqueous solutions with a solubility limit of 17 mg/mL in buffered saline at pH 7.0. It is also soluble in methanol and mixed methanol/water solutions. It is practically insoluble in chloroform.

The QS-21 to be used in this study will be supplied in a vialed sterile aqueous form in Phosphate Buffered Saline (PBS) to be mixed in the clinic with separate vials of antigen.

The QS-21 vial should be resuspended by gentle inversion to assure mixing prior to

withdrawal. A volume of 0.2 ml of the QS-21 formulation should be withdrawn with a sterile 1 mL tuberculin syringe and needle and mixed with the antigen. Although the QS-21 in the vial may have a turbid appearance, it is expected to clarify after dilution with antigen. The QS-21 vials do not contain a preservative, are single-use only, and should not be reentered.

Aqueous QS-21 should be stored at $\leq$ -20ºC; the shelf life at this storage condition is three (3) years. The expiration date is listed on the vials and the certificate of analysis.

A mixed antigen/adjuvant formulation should be used within eight hours of mixing.

**d. Risks/Safety**

Clinical studies containing QS-21 to date have focused primarily on its use as an adjuvant to enhance immune response evoked by preventative as well as therapeutic vaccines against infectious agents and cancers. Studies were and continue to be conducted by many sponsors with a wide variety of antigens and in varying indications.

QS-21 has been evaluated in over 120 different clinical trials of various experimental vaccines in cancer, infectious disease, and neurodegenerative disorders. Mild to moderate pain, erythema and edema at the injection site are common side effects of QS21 containing vaccines. Low-grade fever and severe pain at the injection site may occur, but these effects are uncommon and short-lived. No significant hematological and biological alterations have been documented.

Vaccines containing QS-21 at doses of 50 and 100 µg induce local and systemic side-effects that tend to be more frequent, and of greater intensity compared to the same vaccines in an aluminum hydroxide formulation. However, aluminum hydroxide-induced tissue reactions such as granuloma, necrosis and nodules have never been reported in volunteers administered QS-21 alone, or combined with vaccine antigens. Nearly 90% of volunteers injected with vaccines mixed with 50 or 100 µg of QS-21 experience injection site pain of variable intensity and onset, mild to moderate erythema, induration and some arm soreness. Some volunteers have reported immediate and severe pain on injection following administration of QS-21 preparations. In most studies, this effect occurs in a small percentage of subjects. Data from completed clinical studies indicate these side-effects to be transient, resolving within 7 days without sequelae. Systemic side effects have also been reported following the administration of vaccines combined with 50 µg, 100 µg, and 200 µg of QS-21. The overall incidence and severity of these reactions are generally similar to those of other vaccines, except for a marked increase in reactogenicity among subjects given the 200 µg QS-21 dose. Systemic events reported in recipients of QS-21 formulations include low-grade fever, flu-

like symptoms with body aches, malaise, chills, myalgia, headache, and dizziness. These symptoms are transient, resolving within 48 hours in most subjects. Severe adverse events (vaso-vagal reactions with hypotension and fainting) have also been reported, but they are extremely rare, occurring in less than 1% of QS-21 recipients.

Severe allergic reactions have been noted among recipients of QS-21-containing malaria synthetic peptide vaccines in two trials. Two out of ninety volunteers administered 2 mg of the SPf66 antigen mixed with 50 µg of QS-21 developed generalized pruritus with a few pruritic hives and minor bronchospasm (one volunteer), facial erythema, palpebral edema and dysphonia (one volunteer). These manifestations occurred 5 to 10 minutes after administration of the third vaccine dose, and were accompanied by hypotension. The reactions resolved completely within 60 minutes following systemic therapy with epinephrine, hydrocortisone and anti-allergic drugs. Follow up exams performed 24 hours and 48 hours after the incidents were normal.

Data collected from Phase I and Phase II clinical trials of QS-21 containing vaccines indicate that the vast majority of adverse events are transient reactions confined to the site of injection. The potential health risks associated with these vaccines will depend mainly upon the type of antigen being used, and are likely to be a greater concern if the vaccine antigen has homology with normal tissue constituents. Tumor vaccines admixed with QS-21 should not be administered to persons who have a documented history of autoimmune diseases, unless skin testing or lab results have unequivocally excluded the antigen as a potential source of tissue reaction.

Pregnancy and Lactation

There are no data available on the reproductive effects of QS-21. Therefore, the potential risks posed by QS-21 alone or combined with vaccine antigens are unknown. For this reason, QS-21 formulations should not be given to pregnant or lactating women.

Treatment of Over Dose

No information is available regarding the potential toxicity resulting from an overdose of QS-21 in humans.

**d. <u>Administration</u>**

Vaccine/QS 21 will be administered at weeks 1, 2, 3, and 7, for a total of 4 immunizations for each patient, at rotating sites, which may include the arm, thigh, or abdomen by nurses in the Infusion Center at the ACRC.

**e. Supplier information**

Stimulon® will be supplied by Antigenics, Inc. in vialed sterile aqueous form in phosphate buffered saline (PBS). It will be mixed in the clinic with separate vials of antigen.

**f. Agent Ordering.**
**The drug will be ordered by the pharmacy at UAMS.** *Ask Jennifer for a protocol*.

**g. Agent Accountability**
The vaccine will be stored in the ACRC pharmacy under the supervision of the research pharmacist who will be responsible for maintaining the supply according to the manufacturer's specifications, dispensing the drug for administration and maintenance of all accountability logs. (Standard NCI accountability logs will be used.).

**3.2 Mimotope P10s-PADRE**

**a. Description**

**Peptide Seq:** WRYTAPVHLGDG-aK-Cha-VAAWTLKAAa

Capitol letters – one letter code for L-amino acids
Small letters – one letter code for D-amino acids
Cha - Cyclohexylalanine

PADRE is a synthetic, non-natural Pan HLA-DR binding peptide that binds with high or intermediate affinity to 15 of 16 of the most common HLA-DR types tested to date. Because of its binding promiscuity, PADRE should overcome the problems posed by the extreme polymorphism of HLA-DR molecules in the human population. Furthermore, the PADRE peptide was specifically engineered to be immunogenic in humans. This property represents another significant feature of PADRE, suggesting its potential utility as a carrier to induce T cell "help" in vaccine constructs designed for human use. The safety and immunogenicity of the PADRE peptide, manufactured under cGMP regulations, has been tested in GLP animal studies and in a limited number of Phase 1 clinical trials.

**b. Mechanisms of Action and Toxicology**

To be determined in preclinical study required by the FDA.

**See section e. (risks/safety) for more detailed information on how toxicology and safety parameters will be determined.**

**c. Vaccine Formulation**

NeoMPS Inc (San Diego, CA 92126 · USA) will synthesize P10s mimotope covalently linked with PADRE.
to manufacture the vaccine used in these studies under GMP and stability test (see Appendix material).

**d. Storage and Stability**

To be determined in preclinical studies required by the FDA.

With assistance from NeoMPS, Quality Assurance (QA) and stability and safety plans will be developed. They will include assessments of critical parameters such as potency, purity, sterility and bioburden, pH, and appearance. The objectives are to establish controls for the manufacturing and filling process - controls will include in-process, bulk testing, and final container lot release. Mixing parameters will be established to ensure homogeneity (i.e., that the dosing solution contents are uniform throughout the vial and from vial to vial). Assays for lot release testing, and method(s) for determining vaccine potency will also be established. Test (e.g., bioburden) methods, and criteria for each bulk and filled lot of manufactured vaccine will be defined to obtain a Certificate of Analysis (COA). Criteria and a stability plan will be developed to evaluate vaccine stability under storage and conditions of use during shipping and dose administration

**e. Risks/Safety**

To be determined in preclinical study required by the FDA.

Published studies have shown that at vaccine doses of 50 and 100 µg, antibody and CTL responses against tumor cells are readily induced by peptide P10s-PADRE with no undo harm to mice and immune responses can be persist for 6 months (8, 25, 26, 28, 29, 38).

Technical reports documenting proof of manufacturing integrity, concept studies, assay development, and toxicology assessments will be written and reviewed prior to IND submission.

The preferred animal model for toxicity testing is an animal expressing the relevant tumor antigen. The neolactoseries antigen LeY is not expressed in mice but a structurally related gangliosides, also mimicked by peptide P10s, are endogenously expressed on murine tumors of Balb/c origin. Therefore, a preclinical safety study is being proposed to provide a gross characterization of the nature, frequency, and severity of adverse responses following vaccine administration in this tolerant mouse setting. The preclinical study will provide an initial basis for determining whether the vaccine exhibits a safety

profile appropriate for further study. Groups of animals will be treated with adjuvant only or fixed dose levels of 100, 300, or 500µg (per peptide) of the respective vaccines admixed with 20 µg of QS-21delivered by SC injections. Each treatment will be administered at weeks 1, 2, 3, 7, and 19 for a total of five treatments at each dose, to closely mimic the proposed Phase 1 study. Animals will be monitored three times weekly for injection-site reaction, DTH sensitization (against the immunizing peptide mimotope), and changes in weight or general health status.

For evaluation of immunopathology and serological analyses groups of eight animals will be sacrificed before vaccination and at weeks 3, 9, and 21 for each dose level. Thus, a total of 96 mice will be required for the safety study (vaccine: 8 animals x 3 time points x 3 doses; QS-21: 8 animals x 3 time points). Necropsy will be performed on each animal upon sacrifice or unscheduled death with recording of organ weights and gross pathology and preservation of a complete list of tissues at necropsy. All gross lesions and all tissues from the highest dose will be evaluated and compared with saline-treated animals. Tissues from mice treated at the lower doses will be evaluated by histology only where pathologies are noted in mice treated with 500µg of the vaccine. Dr. Leah Hennings, DVM, an experienced, licensed veterinary pathologist will perform these studies at UAMS. The candidate vaccine will be considered safe if the endpoints meet the following criteria: (i) mortality <5% with no vaccine attributed deaths, (ii) incidence of morbidity <10% with no early sacrifice, (iii) absence of severe injection site reaction (e.g. skin ulceration and/or severe myocyte necrosis), (iv) immunopathology absent or mild in nontarget organs. We will contract with a GLP lab (Clongen Lab LLC, Germantown MD) for limulus assays. If the candidate vaccine exhibits an acceptable safety profile, we will proceed to Phase I studies for evaluation of safety, tolerability and immunogenicity.

**f. <u>Administration</u>**

The **<u>P10s-PADRE Mimotope</u>** will be administered subcutaneously in a volume of up to 1.5 mls in the rotating sites on the abdomen and extremities.

Solubilize 10mg of QS-21 powder directly into the amber glass vial with the appropriate volume of sterile phosphate buffered saline to obtain a stock solution of 2 mg QS-21 per mL
The P10s-PADRE vaccine will be received in powder condition and will be stored frozen at ≤20º C for maximum stability.

6.1 Example of final vaccine solution preparation:

Vaccine stock solution (Ci) = 10mg/mL (or to be determined)
QS-21 stock solution = 2 mg/mL (or to be determined)
Volume needed for 1 mice injection (Vf) = 0.500ml (or to be determined)

| Vaccine concentration mg/ml (Cf) | 2.5 (500µg/dose) | 1.5 (300µg/dose) | 0.5 (100µg/dose) |
|---|---|---|---|

| | | | |
|---|---|---|---|
| Vaccine volume ml (Vi)l | 0.125 | 0.075 | 0.025 |
| QS-21 volume ml (100µg/mouseinjection) | 0.050 | 0.050 | 0.050 |
| Sterile buffered saline volume ml | 0.325 | 0.375 | 0.425 |

Formula for vaccine preparation Vi = (Cf x Vf) / Ci

Add the appropriate amount of sterile buffered saline into the appropriate tubes.
Add the appropriate vaccine volume calculated into the sterile buffered saline.
Mix by voterxing for about 15 seconds.
Add the appropriate volume of QS21 calculated into the diluted vaccine solution.
Mix by gently vortexing the final vaccine solution for about 15 seconds.
Keep on ice until loading the disposable syringe

**g. Label information:**
The vaccine drug supply must be labeled with following:

Caution: New Drug – Limited by Federal law to investigational use.

**h. Supplier Information.**
NeoMPS Inc (San Diego, CA 92126 · USA) will synthesize P10s mimotope covalently linked with PADRE.

**i. Agent Ordering.**
The drug will be ordered by the pharmacy at UAMS.

**j. Agent Accountability**
The vaccine will be stored in the ACRC pharmacy under the supervision of the research pharmacist who will be responsible for maintaining the supply according to the manufacturer's specifications, dispensing the drug for administration and maintenance of all accountability logs. (Standard NCI accountability logs will be used.).

## 4.0 STAGING CRITERIA
The staging will be done according to the American Joint Commission on Cancer (AJCC), sixth edition. The breast cancer staging information can be found at:

http://www.cancerstaging.org/education/tnmschema/breast.ppt#256,1,Slide 1

## 5.0 ELIGIBILITY CRITERIA

**Research Participants:**

Research participants are eligible for the vaccine study if the following inclusion criteria are met:

1) Female research participants of all races with histologically or cytologically confirmed stage IV breast cancer (newly diagnosed, metastatic or relapsed after primary or adjunctive therapy; which has not required a treatment change for 2 months.

2) Age greater than 18 years

3) Karnofsky Performance Status $\geq$ 80.

4) Research participants must not have an active infection requiring treatment with parenteral antibiotics.

5) Research participants must not have other significant medical, surgical or psychiatric conditions or require any medication or treatment which may interfere with compliance of the treatment regimen.

6) Research participants must not have a diagnosis or evidence of organic brain syndrome or significant impairment of basal cognitive function or any psychiatric disorder that might preclude participation in the full protocol.

7) Research participants must have no other current malignancies. Research participants with prior history at any time of any *in situ* cancer, lobular carcinoma of the breast *in situ*, cervical cancer *in situ*, atypical melanocytic hyperplasia or Clark I melanoma *in situ* or basal or squamous skin cancer are eligible, provided they are disease-free at time of registration; patients with other malignancies are eligible if they have been continuously disease free for $\geq$5 years prior to the time of registration.

8) Research participants must not have autoimmune disorders, conditions of immunosuppression or treatment with systemic corticosteroids, including oral steroids (i.e. prednisone, dexamethasone), continuous use of topical steroid creams or ointments, or any steroid containing inhalers. Replacement doses of steroids for patients with adrenal insufficiency are allowed. Research participants who discontinue use of these classes of medication for at least 2 weeks prior to registration are eligible if, in the judgment of the treating physician, the patient is not likely to require these classes of drugs during the treatment period.

9) Research participants must not be allergic to shellfish.

10) Women of childbearing potential must not be pregnant (negative serum pregnancy test within 2 weeks of registration and 48 hours of receiving study drug) or breast-feeding due to the unknown effects of peptide/mimotope vaccines on a fetus.

11) Women of childbearing potential must be counseled to use an accepted and effective method of contraception (including abstinence) while on treatment and for a period of 18 months after completing or discontinuing treatment.

12) Research participants must have WBC $\geq$ 3,000/mm$^3$ and platelet count $\geq$ 100,000/mm$^3$ obtained within 2 weeks prior to registration.

13) Research participants must have SGOT (AST) and bilirubin $\leq$ 2 x institutional upper limit (IUL) of normal and serum creatinine $\leq$ 1.8 mg/dl, all obtained within 2 weeks prior to registration.

14) Research participants must be immunocompetent as measured by responsiveness to a minimum of 2 recall antigens by skin testing. As part of the prestudy skin testing, a mimotope test dose will be administered to rule out hypersensitivity prior to full dose administration.

15) Systemic corticosteroids are not allowed. Research participants who have been on systemic steroids will require a 6 week washout period.

16) All research participants will sign an informed consent approved by the UAMS Institutional Review Board.

17) Prestudy laboratory studies need to be completed within 2 weeks of registration.

## 7.0 TREATMENT PLAN

### 7.1 Treatment Plan and On-study Evaluation.

After signing IRB approved consent the research participants will be assigned to a cohort for dosing at the time of registration in the CRDM by the CRA (see 7.4). All research participants will receive vaccine plus QS 21via sc vaccination following the schedule on the study calendar. Vaccine/QS 21 will be administered at weeks 1, 2, 3, and 7, for a total of 4 immunizations for each patient, at rotating sites, which include the arm, thigh, or abdomen, by nurses in the Infusion Center at the ACRC.

A history and physical examination and toxicity assessment using the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0 will be done prestudy, weeks 1,2,3,7 and 9. A complete blood count with differential, chemistry profile, and screens for autoimmunity will be done at prestudy and weeks 3, 7 and 9. Toxicity evaluations will be done before each injection. Toxicities to be assessed are the laboratory parameters listed in the study calendar as well as any sign or symptom found during the history and physical examination not noted prestudy or on the baseline evaluation with special attention to signs and symptoms related to injection reactions, injection site reactions, or symptoms or laboratory findings indicating autoimmune toxicities. These will be performed according to the study calendar or upon unscheduled presentation due to an adverse event.

Immune response to vaccine will be measured by serologic titer in samples drawn just before each scheduled vaccination on prestudy, weeks 2, 3,and 7. DTH skin testing will be done on weeks 4 and 9 with –P10s-PADRE Mimotope 100 ug given id and Tetanus-Diptheria Toxoid Antigen as a control antigen administered id at a separate location on the back. Induration will be read at 48 hours.

**7.2 Prohibited Medications:**
Systemic steroids are prohibited. If a research participant has been on systemic steroids a 6 week washout will be required.

**7.3 Rescue Medications**
Research participants who develop symptomatic autoimmune reactions, grade 3 or greater hypersensitivitiy reactiond or grade 3 or greater local reactions should be treated as indicated with systemic steroids, topical steroids, epinephrine, or benadryl. These participants will need to be removed from the study (see 8.3).

**7.4 Dose Assignment:**

Research participants will be treated with Vaccine admixed QS 21 on weeks 1, 2, 3, and 7 in cohorts according to following dosing table:

| | **Vaccine Dose** | **QS 21** |
|---|---|---|
| Cohort 1 | 100 μg | 100 μg |
| Cohort 2 | 300 μg | 100 μg |
| Cohort 3 | 500 μg | 100 μg |

The SID will be defined as the lowest observed dose at which four or more out of six patients show an adequate immune response to vaccine. Adequacy of immune response and DLT are both defined in Section 1.4, Endpoints of interest. The decision to escalate the dose, expand the cohort, or terminate the study will be based on assessment for DLT and the adequacy of the immune response, which will require 9 weeks per research participant. The time to assess a cohort of three for DLTs and immune responses is thus anticipated to be 13 weeks based on an accrual rate of two eligible Stage IV patients per month. Upon evaluation of all research participants in a cohort (3-6/dose), dose escalation will proceed according to the schedule shown in Tables 1 and 2.

| Table 1: Toxicity Decision Rules for Dose Escalation Design of Table 2 | |
|---|---|
| ***DLTs/ Cohort***[1] | ***Action*** |
| 0/3 | Use Table 2 to determine next action |
| 1/3 | Expand cohort to 6 subjects |
| 1/6 | Use Table 2 to determine next action |
| 2/6, 3/6, or 4/6 | Stop: Previous dose level is MTD[2] |
| 2/3 or 3/3 | Stop: Previous dose level is MTD[2] |
| [1] DLT = Dose-Limiting Toxicity.<br>[2] MTD = Maximum Tolerated Dose. | |

| Table 2: Dose Escalation Design for SID | |
|---|---|
| ***Success/ Cohort***[1] | ***Action*** |
| 0/3 or 1/3 | Enroll 3 more patients at next higher dose[2] |
| 2/3 or 3/3 | Expand cohort to 6 subjects[2] |
| 2/6 or 3/6 | Enroll 3 more patients at next higher dose[3] |
| 4/6, 5/6, or 6/6 | Stop: SID has been attained[3] |
| [1] Success = Adequate immune response.<br>[2] If no DLTs are observed; otherwise, use Table 1<br>[3] If 0 or 1 DLTs are observed; otherwise, use Table 1 | |

The research participants will be assigned to cohorts by the CRA for the trial who will notify the research nurse who will notify the investigator. Only one cohort will be open to enrollment at a time. The study will have a hold pending the evaluation of each cohort. If two or more subjects are enrolled on the same day, then their injection schedules will be staggered at least one day apart, so that no two subjects in the same cohort commence treatment on the same day. The research pharmacist will be notified of the registration, the cohort dose and the patient number by the CRA. The research participants in a given cohort of 3 can start injections simultaneously. The subsequent cohorts may start after the last research participant in the previous 3 completes the week 9 serology.

## 8.0 RISKS AND TOXICITIES TO BE MONITORED

**Potential toxicities risks benefits and precautions:**

| Procedure | Risks | Measures to Minimize Risks |
|---|---|---|
| Complete History and Physical Exam, Including blood chemistries | Identification of previously unknown condition | Qualified Health Care provider to evaluate potential subject<br>Research records are kept in a locked area with access to study personnel only. |
| Administration of Study Vaccine Mimotope P10s-PADRE | Experimental agent may be toxic or harmful. | Careful monitoring by clinic visits and 24 hour, 7 days per week physicians on call for unexpected problems. |

| | | |
|---|---|---|
| | First time use in humans | Only non-pregnant, non-lactating females may participate. The use of contraception during the study and the use of contraception for 18 months post completion of the trial is required.<br><br>Frequent laboratory test including CBC with differential, liver function tests, etc. |
| | Risks of Local reaction (i.e. swelling, redness, tenderness, itching, extravasations)<br><br>Potential for side effects ranging from hemotologic toxicitites and hypersensitivity reactions to anaphylaxis.<br><br>Unanticipated risks<br><br>Unknown risks | Close and frequent monitoring of participant by qualified staff.<br><br>Test dose/ prescreen DTH to monitor for hypersensitivity reactions<br><br>Emergency equipment at site (including crash carts); ACLS certified staff; Rescue Medications such as Benadryl, epinephrine, high dose steroids, etc.<br><br>The Medical Monitor will review all toxicities on a regular basis and will be available to aid research participants as needed.<br><br>The study drug may be discontinued.<br><br>This is an experienced clinical research center.<br><br>There is a reporting and monitoring mechanism in place for SAE/AE or Unanticipated problems |
| Administration of QS 21 100 µg subcut | Dermatology/Skin: local erythema, rash, pruritis;<br>Gastrointestinal: Diarrhea, anorexia, nausea, vomiting, abnormal taste;<br>Hepatic: Elevated | Careful monitoring by clinic visits and 24 hour, 7 days per week physicians on call for unexpected problems.<br><br>Only non-pregnant, non-lactating females may participate. The use of contraception during the study and the use of contraception for 18 months post completion of the trial is |

| | | |
|---|---|---|
| | hepatic enzymes, hypo-albuminemia with prolonged treatment; Neurology:Confusion, neuropathies; Pulmonary: Dyspnea (due to fluid retention and capillary leak syndrome), pleuritis; Cardiovascular: HTN Cardiac arrhythmias, atrial fibrillation, pericarditis; Pain: Headache, arthralgias, bone pain, abdominal pain, chest pain, myalgia; Coagulation: Partial thromboplastin time (PTT), Prothrombin time (PT), thrombo-embolic phenomena; Fever, flu-like syndrome (chills, rigors, myalgias), fatigue, headache, abnormal labs includind BUN and albumin. | required.<br><br>Frequent laboratory test including CBC with differential, liver function tests, etc.<br><br>Close and frequent monitoring of participant by qualified staff.<br><br>Test dose/ prescreen DTH to monitor for hypersensitivity reactions<br><br>Emergency equipment at site (including crash carts); ACLS certified staff; Rescue Medications such as Benedryl, epinephrine, high dose steroids, etc.<br><br>The Medical Monitor will review all toxicities on a regular basis and will be available to aid research participants as needed.<br><br>The study drug may be discontinued.<br><br>This is an experienced clinical research center.<br><br>There is a reporting and monitoring mechanism in place for SAE/AE or Unanticipated problems |
| Collection of Blood Samples | Pain, bruising at the injection site, and rarely infection.<br><br>Discovery of previously unknown conditions | Experienced personnel will perform the phlebotomies using approved techniques. Pressure and dressings will be used to minimize pain, bruising and infection.<br><br>Research records are kept in a locked area with access to study personnel only.<br><br>Participant study numbers will be used for for |

| | | |
|---|---|---|
| | Possible breach of confidentiality | identification of samples that may be retained for future research (to ensure confidentiality) |
| Serum Pregnancy Testing | Discovery of previously unknown conditions<br><br>Possible breach of confidentiality | Research records are kept in a locked area with access to study personnel only.<br><br>Participants will be identified by study numbers only on all research documents. |
| Serum for immunologic evaluation | Discovery of previously unknown conditions<br><br>Discovery of previously unknown conditions<br><br>Possible breach of confidentiality | Research records are kept in a locked area with access to study personnel only.<br><br>Participants will be identified by study numbers only on all research documents. |
| Skin Test and DTH assay (various locations on subjects<br><br>back) | Pain, bruising at the injection site, and rarely infection. Potential for allergic reaction. anaphylaxis<br><br>Discovery of previously unknown conditions | Experienced personnel will perform the injections using approved techniques. Pressure and dressings will be used to minimize pain, bruising and infection.<br><br>Emergency equipment at site (including crash carts); ACLS certified staff; Rescue Medications such as Benedryl, epinephrine, high dose steroids, etc.<br><br>All antigens proposed not fully addressed in protocol<br><br>Experienced clinical research center.<br><br>Reporting and monitoring mechanism in place for SAE/AE or Unanticipated problems<br><br>Research records are kept in a locked area with access to study personnel only.<br><br>Participants will be identified by study numbers only on all research documents. |

| | | |
|---|---|---|
| | | |
| Collection of data | Breach of Patient privacy and confidentiality | Research records are kept in a locked area with access to study personnel only.<br><br>Participants will be identified by study numbers only on all research documents.<br><br>Investigators to provide certification of completion of human subjects protections training course<br><br>UAMS shall retain the records and reports for 2 years after a marketing application is approved for the drug; or, if an application is not approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and FDA has been so notified. After such time all study records will be destroyed as well as the links between identifiers of the research participants and their research study numbers according to the UAMS record destruction policy (appendix: ??. |

Research participants will continue on vaccine injections for the 4 planned doses unless they withdraw from the study or develop Grade 3 toxicity of any type at which time they will discontinue injections. There will be no dose modifications for toxicity. Special attention to toxicities mediated by autoimmune mechanisms will be given such as: colitis, thyroiditis, or SLE as well as injection site local reactions or allergic reactions. The NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 will be used for toxicity and Serious Adverse Event reporting. A copy of the CTCAE Version 3.0 can be downloaded from the CTEP home page (http://ctep.cancer.gov/reporting/ctc.html). All appropriate treatment areas have access to a copy of the CTCAE Version 3.0.

Any research participant may voluntarily revoke consent and withdraw from the study at any time. A research participant may be terminated early for non-compliance, an unrelated intercurrent illness that may affect assessment or place the patient at risk for adverse events or require systemic steroids, deterioration in performance status so as to make participation a hardship for the research participant or for any reason that the investigator feels it is not in the subject's best interest to continue.

### 8.2 Benefits

As this is the first time use in humans there are no clearly defined benefits to participants of this study, however, this vaccine may potentiate an immune response which could improve median progression-free survival and overall survival of cancer patients.

## 9.0 STUDY CALENDAR

| | Week | | | | | | |
|---|---|---|---|---|---|---|---|
| | Prestudy[5] | 1 | 2 | 3 | 4 | 7 | 9 |
| Vaccination (QS-21) | | x | x | x | | x | |
| Toxicity Notation | | x | x | x | | x | x |
| History/PE | x | x | | x | | x | x |
| CBC with Diff | x | | | x | | x | x |
| SGOT | x | | | x | | x | x |
| Alk Phos | x | | | x | | x | x |
| LDH | x | | | x | | x | x |
| GGT | x | | | x | | x | x |
| Creat | x | | | x | | x | x |
| Calcium | x | | | x | | x | x |
| Albumin | x | | | x | | x | x |
| Amylase | x | | | x | | x | x |
| TSH | x | | | x | | x | x |
| $T_4$ | x | | | x | | x | x |
| ANA | x | | | x | | x | x |
| PT/PTT[6] | x | | | x | | | x |
| Serum pregnancy test[1] | x | | | | | x | |
| Study Lab[2] | x | | x | x | x | x | x |
| Skin Test[3] | x | | | | x[4] | | x[4] |

1. For women of child bearing potential to be done prestudy and within 48 hours prior to dosing. One test may suffice for both.
2. 10 ml serum samples in red top tubes for LeY ELISA and CC assays. Call Dr. Kieber-Emmons lab to pick-up specimen: 526-5930
3. Skin Tests to include the following: Tetanus- Diptheria Toxoid Antigen, Tuberculin, Candida Antigen, Trichophyton Antigen, test dose of mimotope
4. DTH assay with mimotope and Diphtheria-tetanus antigen as control to be read at 48 hours.
5. To be completed within 14 days of registration.
6. Prothrombin time/ partial thromboplastin time.

## 10.0 CRITERIA FOR EVALUATION

### Determination of DLT.

See Section 8.0, Toxicities to be monitored. Any Grade 3 toxicity will be dose limiting.

**Immunological evaluation.** Serum will be collected following the time points of the study calendar, flash frozen in aliquots and stored at -80 C. IgM and IgG titers to LeY

will be evaluated by ELISA. A positive LeY-directed immune response will be defined as an anti-LeY serum antibody titer of 1:40 for a baseline pre-vaccination titer of 0 or a ≥ four fold increase over baseline titer >0 (1,11).Patients will be judged to have had an adequate immune response if they have a positive Le-Y-directed immune response at any one of the five appropriate time points on the study calendar (section 9.0).

**Determination of DTH Responses.**
To evaluate DTH responses, as secondary to the sc injected vaccines, study subjects will be skin-tested according to the time points in the study calendar against the P10s-PADRE mimotope and Tetanus- Diptheria Toxoid Antigen as a control antigen, administered id at a separate location on the patients back. Induration will be measured using calipers and reported in mm across two diameters at 48 h. Greater than 5 mm induration will pe considered positive. Responses will be recorded as negative or positive.

## 11.0 STATISTICAL CONSIDERATIONS

## 11.0 STATISTICAL CONSIDERATIONS

**Sample Size, Study Duration, and General Considerations.**

Because each subject will receive multiple injections at constant dose over an extended period of time, the second and third subject of *the same* dose cohort will be enrolled, as available, before the first subject has finished all scheduled injections. However, if two or more subjects are enrolled on the same day, then their injection schedules will be staggered at least one day apart, so that no two subjects in the same cohort commence treatment on the same day. The decision to escalate the dose, expand the cohort, or terminate the study will be based on assessment for DLT and adequacy of the immune response, which will require 9 weeks per patient. The time to assess a cohort of 3 for DLT and whether the SID has been attained is thus anticipated to be 13 weeks based on an accrual rate of 2 eligible Stage IV patients per month. The SID will be defined as the lowest observed vaccine dose at which four or more out of six patients show an adequate immune response. Assuming no DLTs at all three proposed doses, this study will require a minimum of 9 or maximum of 18 patients. Given the recruitment and immunization/evaluation schedule, we expect this study to be completed in a minimum of 9 months and a maximum of 18 months.

It should be noted that a goal of this aim is to ensure safety of the mimotope vaccine. Because of sample size, these studies only provide a qualitative assessment of vaccine immunogenicity. However, our immunization schedule should favor the generation of antibodies(1,11, 41), leading to an SID determination. A positive LeY-directed immune response will be defined as an anti-LeY serum antibody titer of 1:40 for a baseline pre-vaccination titer of 0 or a ≥4-fold increase for a baseline titer >0 (1,11). A patient will be judged to have had an adequate immune response if they have a positive Le-Y-directed immune response at any one of the five appropriate time points on the study calendar (section 9.0). Upon evaluation of all patients in a cohort (3-6/dose), dose escalation will proceed according to the schedule shown in Tables 1 and 2. Table 1 is essentially the toxicity-based "traditional" design of Storer (49), while Table 2 is the structurally

compatible "Proportion [4/6]" design of Hunsberger *et al*. (50) for molecularly targeted endpoints such as adequacy of immune response. In the absence of DLTs, the design of this dose-escalation trial is the "Proportion [4/6]" design. According to Hunsberger *et al.* (50), the probability that dose escalation will occur with their design is 0.94, 0.52, and 0.15 when the respective rates of adequate immune response are 30%, 60%, and 80%. A secondary endpoint will be a DTH response to mimotope P10s-PADRE.

**Data Analysis Plan**

Toxicity will be graded according to the NCI's Common Terminology Criteria for Adverse Events. All observed toxicities will be enumerated terms of type (organ affected or laboratory determination such as absolute neutrophil count), severity (by NCI Common Toxicity Criteria and nadir or maximum values for the laboratory measures), time of onset (i.e. dose number), duration, and reversibility or outcome. A toxicity of Grade 3 or higher will be scored as a DLT if it is deemed to be related to the vaccine or any of its components. LeY-directed immune titers will be tabulated for each patient at baseline and at indicated time points on the study calendar. Immune titers will be compared to baseline values and scored as Ley-directed immune responses (positive or negative) according to the criteria set forth in Section 1.4.1 (Primary Endpoints). The components of DTH response, namely, the induration in mm across two diameters, will be tabulated by antigen (mimotope vs control) for each patient at indicated time points on the study calendar.

For the analysis to meet the primary objective, the frequency of DLTs and of patients with at least one positive LeY-directed immune response will be used to determine the MTD and SID according to the rules set out in Tables 1 and 2 of Section 7. For the analysis to meet the secondary objective, the difference in longer induration diameters, the difference in shorter induration diameters, and the difference in diameter products (longer x shorter) will be calculated at each time point, and plotted via scatter plot against dose. Medians and quartiles will also be reported for any dose cohort of size six.

Any deviations from the above analysis plan will be reported to the FDA as part of the IND application, along with the reason for the deviation. Inasmuch as the above analysis plan is central to supporting the IND application, any deviation would consist of an addition to the existing plan, not a modification of it.

**Missing, Unused, and Spurious Data.**

Missing data will be treated as missing, and will not be imputed. All data collected will necessarily be to the FDA as part of the IND application, so there will be no unused data. Spurious data will be corrected at the source document according to standard operating procedures drawn up in compliance with Code of Federal Regulations 21CFR58; any data documented as spurious that is unable to be corrected at the source will be treated as missing.

## 12.0 REGISTRATION GUIDELINES

Screening logs will be maintained by the study nurses. Registration will occur after the IRB approved consent is signed and eligibility has been confirmed. The research

participants will be registered in the Clinical Research and Data Management Office (CRDM) and assigned a subject number by the CRA. The study number will be used for identification of the research participant during the study.

## 14.0 DATA SUBMISSION SCHEDULE

Data must be submitted according to protocol requirements for ALL research participants registered, whether or not assigned treatment is administered, including research participants deemed to be ineligible or for whom documentation is inadequate to determine eligibility will generally be deemed ineligible. Data while on study will be collected within 14 days of each visit and entered into the protocol database within 14 days of collection. The database will be done in the CaBIG application, C3D, which houses data on a secure server at the NCI. Data will be entered into Web based CRF's which replicate the paper CRF's attached.

| Forms | Screen | W1 | W2 | W3 | W4 | W7 | W9 |
|---|---|---|---|---|---|---|---|
| Eligibility Checklist (EC) | EC | | | | | | |
| Disease History (DH) | DH | | | | | | |
| Demographics (D) | D | | | | | | |
| Medical History (MH) | MH | | | | | | |
| Prior Therapy (PT) | PT | | | | | | |
| Adverse Event (AE) | AE | AE | AE | AE | AE | AE | AE |
| Biological Markers (BM) | BM | BM | BM | BM | BM | BM | BM |
| Concomitant Medications (CM) | CM | CM | CM | CM | CM | CM | CM |
| Physical Exam (PE) | PE | PE | | PE | | PE | PE |
| Radiology (XR) | XR | | | | | | |
| RECIST (REC) | REC | | | | REC | | |
| Vital Signs (VS) | VS | VS | VS | VS | VS | VS | VS |
| Skin test (ST) | ST | | | | ST | | |

UAMS shall retain the records and reports for 2 years after a marketing application is approved for the drug; or, if an application is not approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and FDA has been so notified. After such time all study records will be destroyed as well as the links between identifiers of the research participants and their research study numbers according to the UAMS record destruction policy: appendix ???.

## 15.0 SPECIAL INSTRUCTIONS

Study lab will be collected in the clinic in a 10 ml red top tube. To pick it up call Dr. Bejahtolah Monzavi-Karbassi at 526-5930.

## 16.0 ETHICAL AND REGULATORY CONSIDERATIONS

The following must be observed to comply with Food and Drug Administration regulations for the conduct and monitoring of clinical investigations; they also represent sound research practice:

All study personnel have completed training in GCP and human subject's protection.

### Recruitment and Informed Consent

Research participants will be recruited from the breast cancer clinics (Medical Oncology and Ladies Oncology Clinics) at the Arkansas Cancer Research Center (ACRC) at the University of Arkansas for Medical Sciences (UAMS) campus. The research participants will be identified by preview of the clinic's schedules for Dr. Hutchins and Dr. Makhoul by the research nurse (Karen Mack, R.N.P.). Prior to any research activities the research participant will be approached for participation by their physician who will discuss the protocol along with risks and potential benefits. A clear statement will be made concerning the voluntary nature of her participation and that her decision will have no effect on her remaining care. The research nurse will follow with a detailed review of the informed consent document. The research participant will be encouraged to have family or friends participate in any or all of the process. The research participant will be given time to ask questions and will be questioned to be certain she understands the information and if she agrees to proceed will sign consent. In general, registration and prestudy work will begin the next business day allowing additional time for the research participant to reflect and request additional questions or withdraw. The consent process will be documented in the medical record. A copy of the informed consent document will be given to the research participant and additional copies sent to the medical records for distribution to the research pharmacy. The original will be filed with the regulatory documents in the CRDM. The consent process occurs in a private exam room or in the private office of the research nurse. There will be no additional recruitment materials. The principles of informed consent are described by Federal Regulatory Guidelines (Federal Register Vol. 46, No. 17, January 27, 1981, part 50) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46). They must be followed to comply with FDA regulations for the conduct and monitoring of clinical investigations.

### Institutional Review

This study will be approved by the UAMS IRB as defined by Federal Regulatory Guidelines (Ref. Federal Register Vol. 46, No. 17, January 27, 1981, part 56) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46).

### Drug Accountability

For each investigational drug, drug disposition (drug receipt, dispensing, transfer or return) will be maintained on the Investigational Drug Accountability Record. Drug

supplies will be kept in a secure, limited access storage area under the recommended storage conditions in the research pharmacy in the ACRC under the direction of the research pharmacist. During the course of the study, the following information will be noted on the Drug Accountability Record; the study # and the initials of the research participant (or should this be the patient #) to whom drug is dispensed, the dose, the date(s) and quantity of drug dispensed to the subject. The balance forward, lot number and recorder's initials. These Drug Accountability Records will be readily available for inspection and are open to FDA inspection at any time.

**Adverse Events:**

See full policy appendix 2.

**Adverse Event (AE)** - Any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure. Each AE is a unique representation of a specific event used for medical documentation and scientific analysis. *[ICH E6 1.2]*

**Serious Adverse Event (SAE)** - Any adverse drug experience occurring at any dose that results in any of the following outcomes: death, a life-threatening adverse drug experience, inpatient hospitalization, or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. FDA requires IND sponsors to report serious adverse events through the expedited reporting system. *[21CFR312.32 (a) ICH E6 1.50 Partially IRB Handbook policy 10.3]*

Any adverse experience which meets reporting guidelines for serious adverse events must be reported to the CRA (clinical research associate) for the study in the CRDM office within 24 hours of knowledge of the event. the CRA will follow the adverse event monitoring plan (appendix 2). All SAE's will be reported to the Co-PI, DOD, FDA, IRB and Medical Monitor according to this plan.
All adverse experiences must also be recorded to the Institutional Review Board within 10 days. All adverse experiences must also be recorded in the appropriate section of the case report form. The report should include, whenever possible, the investigator's written medical judgment as to relationship of the adverse experience to study medications(s) (i.e., "probable", "possible" or "unrelated").

**Monitoring**

The Medical Monitor is Dr. Anne Marie Maddox. See CV, attached.

UAMS is the IND Sponsor. A data monitor (s) will be appointed by the monitoring division of the UAMS Office of Research Support and Regulatory Affairs (RSRA) to assure that the rights and well – being of human subjects are protected and the data are accurate, complete and verifiable from source documents and the trial is conducted in compliance with currently approved protocol/amendments, with good clinical practice (GCP) and the applicable regulatory requirements in accordance with 21 CRF 312.
.

The data monitor will be familiar with the investigational products, the protocol, the informed consent form, any other information provided to the subjects, the standard operating procedures (SOP), GCP and applicable regulatory requirements.

Data monitor(s) will have access to research participant's medical records and other study-related records. The investigator agrees to cooperate with the data monitor (s) and Medical Monitor to ensure that any problems detected in the course of these monitoring visits are resolved. Personal contact between the data monitor, medical monitor and the investigator will be maintained throughout the clinical trial to assure that the investigator is fulfilling his obligations and the facilities used in the clinical trial remain acceptable.

**Pre-investigation site visit:**
Pre-investigation site visit will be performed by the data monitor to inspect the facility where the study is going to be conducted and to assure that the investigator and his/her staff understand the protocol and accept to comply with the current regulations for clinical trial conduct in human subjects (21 CFR 312, 21 CFR 50, 21 CFR 56, 21 CFR 11, 21 CFR 21). He/she will document the IRB approval and generate a special report based on which the accrual of human subjects to the trial can begin.

**Periodic site visits:**
The first visit of the data monitor will occur after the first research participant has completed her treatment as specified by the protocol. Subsequent monitoring visits will take place after enrolling each additional cohort or no longer intervals than every 8 weeks. The data monitor will review the case report forms (CRF's), source data/documents and other trial-related records for accuracy, consistency, and completeness. Enrollment of research participants after meeting eligibility criteria and signing a consent form will be documented. Missing visits, withdrawals and subject recruitment rate will be monitored.

**Investigational products:**
The data monitor will verify that the storage conditions and the investigational drug is being dispensed according the study protocol to eligible patients and that there are accurate records of the receipt, use and return of the investigational product.

**Monitoring report:**
After each monitoring visit (no longer than 8 week intervals) a separate monitoring report will be generated and submitted to the investigator and medical monitor. This report will

include significant findings related to deficiencies and deviations from the protocol, SOPs, GCP, and the applicable regulatory requirements and actions taken to prevent recurrence of the detected deviations. The report will make recommendations for actions to be taken to secure compliance.

The study team including the PI, Co-PI, Biostatistician, Medical Monitor and CRA will meet after each cohort to review adverse events and review monitoring reports in order to make adjustments necessary to protect patients and the integrity of the trial

**Research Participants and Data Safety**

.If three SAE's occur with attribution to the study drug, the trial will be suspended until further review by the medical monitor, PI, sponsor and FDA is completed. This will be accomplished by the study team, the PI, Co-PI, Biostatistician, Medical Monitor and CRA either at the regular meeting or a called meeting due to the SAE's called by the Medical Monitor or the PI.

**Audits**

An audit by the UAMS Office of Research Compliance (ORC) will be scheduled after the completion of the first cohort. The audit will follow the ORC standard auditing procedure.

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## 18.0 APPENDIX

### Appendix 1

### Abbreviations

ACRC – Arkansas Cancer Research Center

ADCC – antibody-dependent cytotoxicity

CBER

CDER

CDC – complement dependent cytotoxicity

CRDM- Clinical Research Data Management

CRF – case report form

CTL – cytotoxic T lymphocyte

DLT – dose limiting toxicity

DTH – delayed type hypersensitivity

ELISA – enzyme-linked immunosorbent assay

FBS – fetal bovine serum

g - grams

HS – human serum

ID – intradermal

IND - investigational new drug

IRB - institutional review board

id - intradermally

KLH – keyhole limpet hemocyanin

LeY – Lewis Y antigen

ORC- Office of Research Compliance

PACT - Patient Advocates for Clinical Trials

PBMCS - peripheral blood mononuclear cells

RSRA – (Office of) Research Support and Regulatory Affairs

SC - subcutaneous

SD – standard deviation

SI - stimulation index

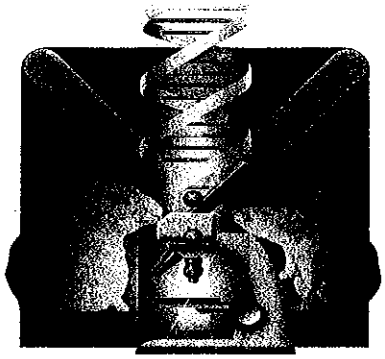
SID – sufficient immunogenic dose

TACA - Tumor associated carbohydrate antigen

UAMS - University of Arkansas for Medical Sciences

µg – Micrograms

Appendix 2: AE policy



# BIOCON Inc.

15801 Crabbs Branch Way Rockville, MD 20855 Phone: 301-417-0585 ~ 800-826-8426

Re: Study 5368-001

April 9, 2008

Dear Dr. Artaud:

I enclose the clinical lab data, tissues and frozen sera from your study. The attached worksheet matches animal identifiers to study group.

In brief, animals entered the facility, were inoculated then observed daily throughout the study in life. There were no indications of any negative effects of your vaccine on general animal welfare or behavior. In addition, there were no negative findings during necropsy.

Good luck in your future endeavors.

Francis W. Klotz

Francis W. Klotz Ph.D.
Director of Toxicology and Pre-Clinical Services
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Rockville, MD 20855
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(301) 921-0168 or (FAX) 977-0433

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|---|---|---|---|
| CLIENT: | BIOCON, INC. | DATE SPEC. COLLECTED: | 03/17/2008 |
| SPECIMEN ID: | J796 | DATE SPEC. RECEIVED: | 03/17/2008 |
| SPECIES: | RABBIT | DATE REPORTED: | 03/19/2008 |
| STUDY: | 5368-001 | AGE: | |
| GROUP: | 1 | SEX: | F |
| ACCESSION: | E 0007506 | | |

| PROFILE/TEST | RESULTS | UNITS | REFERENCE RANGE |
|---|---|---|---|
| **CBC WET** | | | |
| WHITE BLOOD COUNT | 6.4 | THSN/UL | 6.3 - 13.0 |
| RED BLOOD COUNT | 5.39 | MILL/UL | 5.59 - 6.74 |
| HEMOGLOBIN | 11.6 | GM/DL | 11.6 - 14.6 |
| HEMATOCRIT | 35.2 | PERCENT | 38.0 - 45.5 |
| MCV | 65 | FL | 64.7 - 73.3 |
| MCH | 21.5 | PICO GM | 20.2 - 23.4 |
| MCHC | 32.9 | PERCENT | 29.7 - 32.8 |
| RDW | 12.4 | % | - |
| PLATELET | 275 | THSN/UL | 221 - 463 |
| MPV | 6.3 | FL | - |
| **DIFF. (ABSOLUTE)** | | | |
| BANDS | 0.00 | THSN/UL | 0.00 - 0.20 |
| SEGMENTED NEUTROPHILS | 1.15 | THSN/UL | 0.00 - 5.00 |
| LYMPHOCYTES | 5.12 | THSN/UL | 2.00 - 10.00 |
| MONOCYTES | 0.00 | THSN/UL | 0.00 - 1.30 |
| EOSINOPHILS | 0.06 | THSN/UL | 0.00 - 0.45 |
| BASOPHILS | 0.06 | THSN/UL | 0.00 - 0.65 |
| ATYPICAL LYMPHOCYTES | 0.00 | THSN/UL | 0.00 - 0.00 |
| METAMYELOCYTES | 0.00 | THSN/UL | 0.00 - 0.00 |
| MYELOCYTES | 0.00 | THSN/UL | 0.00 - 0.00 |
| NRBC/100 WBC | 0 | /100 WBC | 0 - 0 |
| **U/A (MACROSCOPIC)** | | | |
| COLOR | * | | YELLOW |
| * NO SPECIMEN RECEIVED. | | | |
| **U/A (MICROSCOPIC)** | | | |
| WBC | * | /HPF | |
| * NO SPECIMEN RECEIVED. | | | |
| **BIOCON** | | | |
| SODIUM | 140 | MEQ/L | 139 - 149 |
| POTASSIUM | 6.0 | MEQ/L | 4.8 - 6.1 |
| CHLORIDE | 102 | MEQ/L | 103 - 114 |
| BICARBONATE | 19 | MEQ/L | - - - |
| CALCIUM, TOTAL | 16.2 | MG/DL | 13.4 - 15.4 |
| PHOSPHORUS | 5.8 | MG/DL | 3.9 - 5.9 |
| MAGNESIUM | 2.5 | MEQ/L | 1.6 - 2.8 |
| CREATINE PHOSPHOKINASE | 294 | U/L | 120 - 230 |
| LDH | 79 | U/L | 70 - 130 |
| AST (SGOT) | 18 | U/L | 20 - 75 |

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| SPECIMEN ID: | J796 | DATE SPEC. RECEIVED: | 03/17/2008 |
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| STUDY: | 5368-001 | AGE: | |
| GROUP: | 1 | SEX: | F |
| ACCESSION: | E 0007506 | | |

| PROFILE/TEST | RESULTS | UNITS | REFERENCE RANGE |
|---|---|---|---|
| BIOCON (Continued) | | | |
| ALT (SGPT) | 68 | U/L | 9 - 89 |
| ALP | 149 | U/L | - - - |
| GAMMA GLUTAMYL TRANSFERASE | 10 | U/L | - - - |
| AMYLASE | 261 | U/L | 165 - 533 |
| GLUCOSE | 126 | MG/DL | 93 - 129 |
| BUN | 22 | MG/DL | 15 - 26 |
| CREATININE | 1.1 | MG/DL | 0.8 - 2.4 |
| CHOLESTEROL | 62 | MG/DL | 11 - 85 |
| TRIGLYCERIDE | 37 | MG/DL | 104 - 140 |
| BILIRUBIN, TOTAL | 0.1 | MG/DL | 0.0 - 0.4 |
| BILIRUBIN, DIRECT | 0.0 | MG/DL | 0.0 - 0.2 |
| URIC ACID | 0.4 | MG/DL | 0.9 - 1.5 |
| ALBUMIN | 2.9 | G/DL | 2.7 - 3.7 |
| PROTEIN, TOTAL | 5.7 | G/DL | 4.8 - 9.5 |
| GLOBULIN | 2.8 | G/DL | 2.1 - 5.8 |
| SORBITOL DEHYDROGENASE | 5 | U/L | - - - |

Laboratory Director: Saroj R. Das, Ph.D.



FINAL REPORT

MA 3/20/08

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| GROUP: | 1 | SEX: | F |
| ACCESSION: | E 0007507 | | |

| PROFILE/TEST | RESULTS | UNITS | REFERENCE RANGE |
|---|---|---|---|
| CBC WET | | | |
| WHITE BLOOD COUNT | 6.6 | THSN/UL | 6.3 - 13.0 |
| RED BLOOD COUNT | 5.96 | MILL/UL | 5.59 - 6.74 |
| HEMOGLOBIN | 12.7 | GM/DL | 11.6 - 14.6 |
| HEMATOCRIT | 39.2 | PERCENT | 38.0 - 45.5 |
| MCV | 66 | FL | 64.7 - 73.3 |
| MCH | 21.4 | PICO GM | 20.2 - 23.4 |
| MCHC | 32.5 | PERCENT | 29.7 - 32.8 |
| RDW | 13.7 | % | - |
| PLATELET | 279 | THSN/UL | 221 - 463 |
| MPV | 6.5 | FL | - |
| DIFF. (ABSOLUTE) | | | |
| BANDS | 0.00 | THSN/UL | 0.00 - 0.20 |
| SEGMENTED NEUTROPHILS | 1.06 | THSN/UL | 0.00 - 5.00 |
| LYMPHOCYTES | 5.02 | THSN/UL | 2.00 - 10.00 |
| MONOCYTES | 0.07 | THSN/UL | 0.00 - 1.30 |
| EOSINOPHILS | 0.07 | THSN/UL | 0.00 - 0.45 |
| BASOPHILS | 0.40 | THSN/UL | 0.00 - 0.65 |
| ATYPICAL LYMPHOCYTES | 0.00 | THSN/UL | 0.00 - 0.00 |
| METAMYELOCYTES | 0.00 | THSN/UL | 0.00 - 0.00 |
| MYELOCYTES | 0.00 | THSN/UL | 0.00 - 0.00 |
| NRBC/100 WBC | 0 | /100 WBC | 0 - 0 |
| U/A (MACROSCOPIC) | | | |
| COLOR | YELLOW | | YELLOW |
| APPEARANCE | TURBID | | CLEAR |
| SPECIFIC GRAVITY | 1.040 | SG UNITS | 1.003 - 1.036 |
| GLUCOSE | 0 | MG/DL | - |
| BILIRUBIN | 0.0 | # PLUS | - |
| KETONES | 0.0 | MG/DL | - |
| OCCULT BLOOD | 0.0 | # PLUS | - |
| pH | 8.5 | pH UNITS | 7.6 - 8.8 |
| UR PROTEIN | 30 | MG/DL | - |
| UROBILINOGEN | 0.2 | MG/DL | - |
| U/A (MICROSCOPIC) | | | |
| BACTERIA | TRACE | /HPF | |
| CRYSTALS | 3+ CA CARB | /HPF | |

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| STUDY: | 5368-001 | AGE: | |
| GROUP: | 1 | SEX: | F |
| ACCESSION: | E 0007507 | | |

| PROFILE/TEST | RESULTS | UNITS | REFERENCE RANGE |
|---|---|---|---|
| BIOCON | | | |
| SODIUM | 140 | MEQ/L | 139 - 149 |
| POTASSIUM | 6.1 | MEQ/L | 4.8 - 6.1 |
| CHLORIDE | 104 | MEQ/L | 103 - 114 |
| BICARBONATE | 19 | MEQ/L | - - - |
| CALCIUM, TOTAL | 16.2 | MG/DL | 13.4 - 15.4 |
| PHOSPHORUS | 6.8 | MG/DL | 3.9 - 5.9 |
| MAGNESIUM | 2.9 | MEQ/L | 1.6 - 2.8 |
| CREATINE PHOSPHOKINASE | 311 | U/L | 120 - 230 |
| LDH | 59 | U/L | 70 - 130 |
| AST (SGOT) | 14 | U/L | 20 - 75 |
| ALT (SGPT) | 68 | U/L | 9 - 89 |
| ALP | 351 | U/L | - - - |
| GAMMA GLUTAMYL TRANSFERASE | 16 | U/L | - - - |
| AMYLASE | 247 | U/L | 165 - 533 |
| GLUCOSE | 136 | MG/DL | 93 - 129 |
| BUN | 21 | MG/DL | 15 - 26 |
| CREATININE | 1.1 | MG/DL | 0.8 - 2.4 |
| CHOLESTEROL | 75 | MG/DL | 11 - 85 |
| TRIGLYCERIDE | 67 | MG/DL | 104 - 140 |
| BILIRUBIN, TOTAL | 0.0 | MG/DL | 0.0 - 0.4 |
| BILIRUBIN, DIRECT | 0.0 | MG/DL | 0.0 - 0.2 |
| URIC ACID | 0.3 | MG/DL | 0.9 - 1.5 |
| ALBUMIN | 4.1 | G/DL | 2.7 - 3.7 |
| PROTEIN, TOTAL | 5.7 | G/DL | 4.8 - 9.5 |
| GLOBULIN | 1.6 | G/DL | 2.1 - 5.8 |
| SORBITOL DEHYDROGENASE | 3 | U/L | - - - |

Laboratory Director: Saroj R. Das, Ph.D.



FINAL REPORT

3/20/08

ANI LYTICS, INC.
200 GIRARD STREET
SUITE 200
GAITHERSBURG, MD 20877
(301) 921-0168 or (FAX) 977-0433

| | | | |
|---|---|---|---|
| CLIENT: | BIOCON, INC. | DATE SPEC. COLLECTED: | 03/17/2008 |
| SPECIMEN ID: | J798 | DATE SPEC. RECEIVED: | 03/17/2008 |
| SPECIES: | RABBIT | DATE REPORTED: | 03/19/2008 |
| STUDY: | 5368-001 | AGE: | |
| GROUP: | 2 | SEX: | F |
| ACCESSION: | E 0007508 | | |

| PROFILE/TEST | RESULTS | UNITS | REFERENCE RANGE |
|---|---|---|---|
| CBC WET | | | |
| WHITE BLOOD COUNT | 6.7 | THSN/UL | 6.3 - 13.0 |
| RED BLOOD COUNT | 5.48 | MILL/UL | 5.59 - 6.74 |
| HEMOGLOBIN | 12.2 | GM/DL | 11.6 - 14.6 |
| HEMATOCRIT | 37.4 | PERCENT | 38.0 - 45.5 |
| MCV | 68 | FL | 64.7 - 73.3 |
| MCH | 22.4 | PICO GM | 20.2 - 23.4 |
| MCHC | 32.7 | PERCENT | 29.7 - 32.8 |
| RDW | 13.7 | % | - |
| PLATELET | 287 | THSN/UL | 221 - 463 |
| MPV | 6.4 | FL | - |
| DIFF. (ABSOLUTE) | | | |
| BANDS | 0.00 | THSN/UL | 0.00 - 0.20 |
| SEGMENTED NEUTROPHILS | 2.48 | THSN/UL | 0.00 - 5.00 |
| LYMPHOCYTES | 3.48 | THSN/UL | 2.00 - 10.00 |
| MONOCYTES | 0.13 | THSN/UL | 0.00 - 1.30 |
| EOSINOPHILS | 0.13 | THSN/UL | 0.00 - 0.45 |
| BASOPHILS | 0.47 | THSN/UL | 0.00 - 0.65 |
| ATYPICAL LYMPHOCYTES | 0.00 | THSN/UL | 0.00 - 0.00 |
| METAMYELOCYTES | 0.00 | THSN/UL | 0.00 - 0.00 |
| MYELOCYTES | 0.00 | THSN/UL | 0.00 - 0.00 |
| NRBC/100 WBC | 0 | /100 WBC | 0 - 0 |
| U/A (MACROSCOPIC) | | | |
| COLOR | YELLOW | | YELLOW |
| APPEARANCE | TURBID | | CLEAR |
| SPECIFIC GRAVITY | 1.044 | SG UNITS | 1.003 - 1.036 |
| GLUCOSE | 0 | MG/DL | - |
| BILIRUBIN | 0.0 | # PLUS | - |
| KETONES | 0.0 | MG/DL | - |
| OCCULT BLOOD | 0.0 | # PLUS | - |
| pH | 8.5 | pH UNITS | 7.6 - 8.8 |
| UR PROTEIN | 30 | MG/DL | - |
| UROBILINOGEN | 0.2 | MG/DL | - |
| U/A (MICROSCOPIC) | | | |
| BACTERIA | TRACE | /HPF | |
| CRYSTALS | 3+ CA CARB | /HPF | |

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| CLIENT: | BIOCON, INC. | DATE SPEC. COLLECTED: | 03/17/2008 |
| SPECIMEN ID: | J798 | DATE SPEC. RECEIVED: | 03/17/2008 |
| SPECIES: | RABBIT | DATE REPORTED: | 03/19/2008 |
| STUDY: | 5368-001 | AGE: | |
| GROUP: | 2 | SEX: | F |
| ACCESSION: | E 0007508 | | |

| PROFILE/TEST | RESULTS | UNITS | REFERENCE RANGE |
|---|---|---|---|
| BIOCON | | | |
| SODIUM | 141 | MEQ/L | 139 - 149 |
| POTASSIUM | 5.9 | MEQ/L | 4.8 - 6.1 |
| CHLORIDE | 107 | MEQ/L | 103 - 114 |
| BICARBONATE | 20 | MEQ/L | - - - |
| CALCIUM, TOTAL | 14.7 | MG/DL | 13.4 - 15.4 |
| PHOSPHORUS | 5.0 | MG/DL | 3.9 - 5.9 |
| MAGNESIUM | 2.8 | MEQ/L | 1.6 - 2.8 |
| CREATINE PHOSPHOKINASE | 181 | U/L | 120 - 230 |
| LDH | 93 | U/L | 70 - 130 |
| AST (SGOT) | 12 | U/L | 20 - 75 |
| ALT (SGPT) | 50 | U/L | 9 - 89 |
| ALP | 152 | U/L | - - - |
| GAMMA GLUTAMYL TRANSFERASE | 18 | U/L | - - - |
| AMYLASE | 280 | U/L | 165 - 533 |
| GLUCOSE | 136 | MG/DL | 93 - 129 |
| BUN | 18 | MG/DL | 15 - 26 |
| CREATININE | 1.0 | MG/DL | 0.8 - 2.4 |
| CHOLESTEROL | 47 | MG/DL | 11 - 85 |
| TRIGLYCERIDE | 56 | MG/DL | 104 - 140 |
| BILIRUBIN, TOTAL | 0.0 | MG/DL | 0.0 - 0.4 |
| BILIRUBIN, DIRECT | 0.0 | MG/DL | 0.0 - 0.2 |
| URIC ACID | 0.5 | MG/DL | 0.9 - 1.5 |
| ALBUMIN | 2.9 | G/DL | 2.7 - 3.7 |
| PROTEIN, TOTAL | 5.4 | G/DL | 4.8 - 9.5 |
| GLOBULIN | 2.5 | G/DL | 2.1 - 5.8 |
| SORBITOL DEHYDROGENASE | 4 | U/L | - - - |

Laboratory Director: Saroj R. Das, Ph.D.



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ANI LYTICS, INC.
200 GIRARD STREET
SUITE 200
GAITHERSBURG, MD 20877
(301) 921-0168 or (FAX) 977-0433

| | | | |
|---|---|---|---|
| CLIENT: | BIOCON, INC. | DATE SPEC. COLLECTED: | 03/17/2008 |
| SPECIMEN ID: | J799 | DATE SPEC. RECEIVED: | 03/17/2008 |
| SPECIES: | RABBIT | DATE REPORTED: | 03/19/2008 |
| STUDY: | 5368-001 | AGE: | |
| GROUP: | 2 | SEX: | F |
| ACCESSION: | E 0007509 | | |

| PROFILE/TEST | RESULTS | UNITS | REFERENCE RANGE |
|---|---|---|---|
| CBC WET | | | |
| WHITE BLOOD COUNT | 7.1 | THSN/UL | 6.3 - 13.0 |
| RED BLOOD COUNT | 5.93 | MILL/UL | 5.59 - 6.74 |
| HEMOGLOBIN | 12.1 | GM/DL | 11.6 - 14.6 |
| HEMATOCRIT | 37.4 | PERCENT | 38.0 - 45.5 |
| MCV | 63 | FL | 64.7 - 73.3 |
| MCH | 20.4 | PICO GM | 20.2 - 23.4 |
| MCHC | 32.4 | PERCENT | 29.7 - 32.8 |
| RDW | 14.5 | % | - |
| PLATELET | 440 | THSN/UL | 221 - 463 |
| MPV | 6.1 | FL | - |
| DIFF. (ABSOLUTE) | | | |
| BANDS | 0.00 | THSN/UL | 0.00 - 0.20 |
| SEGMENTED NEUTROPHILS | 3.12 | THSN/UL | 0.00 - 5.00 |
| LYMPHOCYTES | 3.48 | THSN/UL | 2.00 - 10.00 |
| MONOCYTES | 0.14 | THSN/UL | 0.00 - 1.30 |
| EOSINOPHILS | 0.07 | THSN/UL | 0.00 - 0.45 |
| BASOPHILS | 0.28 | THSN/UL | 0.00 - 0.65 |
| ATYPICAL LYMPHOCYTES | 0.00 | THSN/UL | 0.00 - 0.00 |
| METAMYELOCYTES | 0.00 | THSN/UL | 0.00 - 0.00 |
| MYELOCYTES | 0.00 | THSN/UL | 0.00 - 0.00 |
| NRBC/100 WBC | 0 | /100 WBC | 0 - 0 |
| U/A (MACROSCOPIC) | | | |
| COLOR | YELLOW | | YELLOW |
| APPEARANCE | TURBID | | CLEAR |
| SPECIFIC GRAVITY | 1.030 | SG UNITS | 1.003 - 1.036 |
| GLUCOSE | 0 | MG/DL | - |
| BILIRUBIN | 0.0 | # PLUS | - |
| KETONES | 0.0 | MG/DL | - |
| OCCULT BLOOD | 0.0 | # PLUS | - |
| pH | 8.5 | pH UNITS | 7.6 - 8.8 |
| UR PROTEIN | 30 | MG/DL | - |
| UROBILINOGEN | 0.2 | MG/DL | - |
| U/A (MICROSCOPIC) | | | |
| BACTERIA | TRACE | /HPF | |
| CRYSTALS | 3+ CA CARB | /HPF | |

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| CLIENT: | BIOCON, INC. | DATE SPEC. COLLECTED: | 03/17/2008 |
| SPECIMEN ID: | J799 | DATE SPEC. RECEIVED: | 03/17/2008 |
| SPECIES: | RABBIT | DATE REPORTED: | 03/19/2008 |
| STUDY: | 5368-001 | AGE: | |
| GROUP: | 2 | SEX: | F |
| ACCESSION: | E 0007509 | | |

| PROFILE/TEST | RESULTS | UNITS | REFERENCE RANGE |
|---|---|---|---|
| BIOCON | | | |
| SODIUM | 144 | MEQ/L | 139 - 149 |
| POTASSIUM | 6.4 | MEQ/L | 4.8 - 6.1 |
| CHLORIDE | 107 | MEQ/L | 103 - 114 |
| BICARBONATE | 20 | MEQ/L | - - - |
| CALCIUM, TOTAL | 16.9 | MG/DL | 13.4 - 15.4 |
| PHOSPHORUS | 5.4 | MG/DL | 3.9 - 5.9 |
| MAGNESIUM | 2.8 | MEQ/L | 1.6 - 2.8 |
| CREATINE PHOSPHOKINASE | 173 | U/L | 120 - 230 |
| LDH | 52 | U/L | 70 - 130 |
| AST (SGOT) | 8 | U/L | 20 - 75 |
| ALT (SGPT) | 41 | U/L | 9 - 89 |
| ALP | 105 | U/L | - - - |
| GAMMA GLUTAMYL TRANSFERASE | 12 | U/L | - - - |
| AMYLASE | 507 | U/L | 165 - 533 |
| GLUCOSE | 138 | MG/DL | 93 - 129 |
| BUN | 23 | MG/DL | 15 - 26 |
| CREATININE | 1.0 | MG/DL | 0.8 - 2.4 |
| CHOLESTEROL | 56 | MG/DL | 11 - 85 |
| TRIGLYCERIDE | 79 | MG/DL | 104 - 140 |
| BILIRUBIN, TOTAL | 0.0 | MG/DL | 0.0 - 0.4 |
| BILIRUBIN, DIRECT | 0.0 | MG/DL | 0.0 - 0.2 |
| URIC ACID | 0.4 | MG/DL | 0.9 - 1.5 |
| ALBUMIN | 3.1 | G/DL | 2.7 - 3.7 |
| PROTEIN, TOTAL | 6.1 | G/DL | 4.8 - 9.5 |
| GLOBULIN | 3.0 | G/DL | 2.1 - 5.8 |
| SORBITOL DEHYDROGENASE | 4 | U/L | - - - |

Laboratory Director: Saroj R. Das, Ph.D.



FINAL REPORT

MH 3/20/08